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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

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in its capacity as elected Office

Date of mailing (day/month/year)
25 January 2000 (25.01.00)

International application No.
PCT/GB99/01824

Applicant's or agent's file reference
KMN/FP5780044

International filing date (day/month/year)
09 June 1999 (09.06.99)

Priority date (day/month/year)
10 June 1998 (10.06.98)

Applicant

MATASSA, Victor et al

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
15 December 1999 (15.12.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Olivia RANAIVOJAONA

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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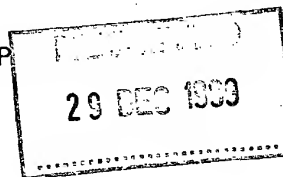
NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

From the INTERNATIONAL BUREAU

To:

NICHOLLS, Kathryn, M.
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| Date of mailing (day/month/year) 16 December 1999 (16.12.99) | | IMPORTANT NOTICE | |
| Applicant's or agent's file reference KMN/FP5780044 | | | |
| International application No. PCT/GB99/01824 | International filing date (day/month/year) 09 June 1999 (09.06.99) | Priority date (day/month/year) 10 June 1998 (10.06.98) | |
| Applicant al ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE P ANGELETTI S.P.A. et | | | |

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
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In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
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The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
16 December 1999 (16.12.99) under No. WO 99/64442

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a **demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

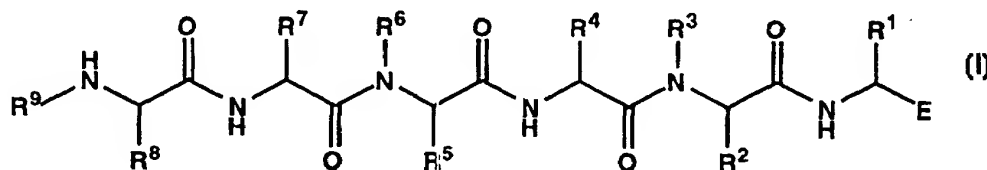
For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

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| The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland | Authorized officer J. Zahra |
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(54) Title: ANTIVIRAL PEPTIDE DERIVATIVES



(57) Abstract

The invention provides amino acid derivatives of formula (I) wherein E represents CHO or B(OH)₂; R¹ represents lower alkyl (optionally substituted by halo, cyano, lower alkylthio, aryl-lower alkylthio, aryl or heteroaryl), lower alkenyl or lower alkynyl; R² represents lower alkyl optionally substituted by hydroxy, carboxy, aryl, aminocarbonyl or lower cycloalkyl; and R³ represents hydrogen or lower alkyl; or R² and R³ together represent di- or trimethylene optionally substituted by hydroxy; R⁴ represents lower alkyl (optionally substituted by hydroxy, lower cycloalkyl, carboxy, aryl, lower alkylthio, cyano-lower alkylthio or aryl-lower alkylthio), lower alkenyl, aryl or lower cycloalkyl; R⁵ represents lower alkyl (optionally substituted by hydroxy, lower alkylthio, aryl, aryl-lower alkylthio or cyano-lower alkylthio) or lower cycloalkyl; R⁶ represents hydrogen or lower alkyl; R⁷ represents lower alkyl (optionally substituted by hydroxy, carboxy, aryl or lower cycloalkyl) or lower cycloalkyl; R⁸ represents lower alkyl optionally substituted by hydroxy, carboxy or aryl; and R⁹ represents lower alkylcarbonyl, carboxy-lower alkylcarbonyl, arylcarbonyl, lower alkylsulphonyl, arylsulphonyl, lower alkoxy carbonyl or aryl-lower alkoxy carbonyl, and salts of acidic compounds of formula (I) with bases, which are viral proteinase inhibitors useful as antiviral agents, especially for the treatment or prophylaxis of infections caused by Hepatitis C, Hepatitis G and human GB viruses.

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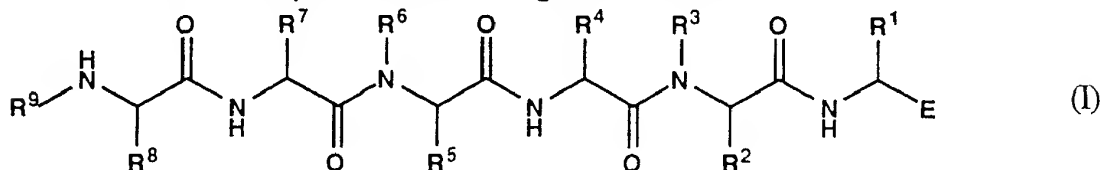
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ANTIVIRAL PEPTIDE DERIVATIVES

The present invention is concerned with amino acid derivatives and a process for their manufacture.

10 The amino acid derivatives provided by the present invention are compounds of the general formula



wherein

- E represents CHO or B(OH)₂;
- 15 R¹ represents lower alkyl, halo-lower alkyl, cyano-lower alkyl, lower alkylthio-lower alkyl, aryl-lower alkylthio-lower alkyl, aryl-lower alkyl, heteroaryl-lower alkyl, lower alkenyl or lower alkynyl;
- R² represents lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, aminocarbonyl-lower alkyl or lower cycloalkyl-lower alkyl; and
- 20 R³ represents hydrogen or lower alkyl; or
- R² and R³ together represent di- or trimethylene optionally substituted by hydroxy;
- 25 R⁴ represents lower alkyl, hydroxy-lower alkyl, lower cycloalkyl-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, lower alkylthio-lower alkyl, cyano-lower alkylthio-lower alkyl, aryl-lower alkylthio-lower alkyl, lower alkenyl, aryl or lower cycloalkyl;
- 30 R⁵ represents lower alkyl, hydroxy-lower alkyl, lower alkylthio-lower alkyl, aryl-lower alkyl, aryl-lower alkylthio-lower alkyl, cyano-lower alkylthio-lower alkyl or lower cycloalkyl;
- R⁶ represents hydrogen or lower alkyl;
- 35 R⁷ represent lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, lower cycloalkyl-lower

- alkyl or lower cycloalkyl;
- R⁸ represents lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl or aryl-lower alkyl; and
- R⁹ represents lower alkylcarbonyl, carboxy-lower alkylcarbonyl, arylcarbonyl, lower alkylsulphonyl, arylsulphonyl, lower alkoxycarbonyl or aryl-lower alkoxycarbonyl;

and salts of acidic compounds of formula I with bases.

- 10 The compounds of formula I and their aforementioned salts inhibit proteinases of viral origin and are useful in the treatment of viral infections, particularly viral infections caused by Hepatitis C, Hepatitis G and the human GB viruses.

- 15 As used in this specification, the term "lower alkyl", alone or in combination, denotes a straight-chain or branched chain alkyl group preferably containing 1-7, especially 1-4, carbon atoms, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec.butyl, tert.butyl, n-pentyl, neopentyl and the like. "The terms
- 20 "lower alkenyl" and "lower alkynyl" denote alkenyl groups preferably containing 2-7 carbon atoms, e.g. vinyl, allyl, n-propenyl, n-butenyl, and the like, and, respectively, alkynyl groups preferably containing 2-7 carbon atoms, e.g. propargyl and the like. The term "lower cycloalkyl" denotes a cycloalkyl group
- 25 preferably containing 3-7 carbon atoms, e.g. cyclopropyl, cyclobutyl, cyclopentyl and the like. The lower alkoxy part of a "lower alkoxycarbonyl" group is preferably a lower alkyl ether group in which the lower alkyl moiety has the aforementioned significance. The term "aryl" denotes a monocyclic or polycyclic
- 30 aromatic hydrocarbon group, e.g. phenyl, naphthyl or the like which is unsubstituted or substituted by one or more substituents selected from e.g. lower alkyl, lower alkoxy, nitro, halo, halo-lower alkyl, hydroxy, acetamido and the like. The term
- "heteroaryl" denotes a 5- or 6-membered aromatic heterocyclic
- 35 group which contains N, O and/or S as the hetero atom(s) and which is optionally benz-fused and/or substituted in the same manner as the aryl group defined above. Examples of heteroaryl groups are furyl, thienyl, pyridyl, pyrimidinyl, benzofuranyl,

benzothienyl, quinolyl, isoquinolyl and the like.

The compounds of formula I contain at least six asymmetric carbon atoms and can therefore exist in the form of optically pure diastereoisomers, mixtures of diastereoisomers, diastereoisomeric racemates or mixtures of diastereoisomeric racemates. The present invention includes within its scope all of these possible forms.

One class of preferred compounds of formula I comprises those in which R¹ represents lower alkyl, halo-lower alkyl, lower alkylthio-lower alkyl, aryl-lower alkylthio-lower alkyl, hetero-aryl-lower alkyl, lower alkenyl or lower alkynyl. Fluoro-lower alkyl is the preferred halo-lower alkyl group. Preferred hetero-aryl-lower alkyl groups are thienyl-lower alkyl and furyl-lower alkyl. Preferably, R² represents lower alkyl, lower cycloalkyl-lower alkyl or aryl-lower alkyl and R³ represents hydrogen or R² and R³ together represent trimethylene optionally substituted by hydroxy. R⁴ preferably represents lower alkyl, lower cycloalkyl-lower alkyl, aryl-lower alkyl, aryl or lower cycloalkyl, R⁵ preferably represents aryl-lower alkyl or lower cycloalkyl, R⁶ preferably represents hydrogen, R⁷ preferably represents lower alkyl, carboxy-lower alkyl, aryl-lower alkyl or hydroxy-lower alkyl, R⁸ preferably represents hydroxy-lower alkyl, carboxy-lower alkyl or aryl-lower alkyl and R⁹ preferably represents lower alkylcarbonyl or carboxy-lower alkylcarbonyl.

Examples of these preferred compounds in which E represents CHO are:

30

2(S)-[[N-[N-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butyraldehyde;

2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4-difluorovaleraldehyde;

2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-

- leucyl]amino]-4,4,4-trifluorobutyraldehyde;
2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(methylthio)propionaldehyde;
5 2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(butylthio)propionaldehyde;
2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentenaldehyde;
10 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentynal;
2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexynal;
15 3-(benzylthio)-2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propionaldehyde;
20 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(2-thienyl)propionaldehyde;
2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(3-thienyl)propionaldehyde;
25 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-3-(2-naphthyl)-D-alanyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L-seryl-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
30 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]hexanal;
35 (Z)-2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexenal;
2(RS)-[[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-L-

- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-L-phenylalanyl]-3-methyl-L-valyl]-L-
 5 leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L-
 10 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-methylhexanal;
 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-hexenal;
 15 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-norleucyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-2-cyclohexylglycyl]-2-methyl-L-phenylalanyl]-3-methyl-L-
 20 valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde; and
 2(RS)-[[N-[N-[N-[N-(4-acetamidobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;

- 25 and examples of these preferred compounds in which E represents B(OH)₂ are:

- 1(RS)-[[N-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-
 30 leucyl]amino]propylboronic acid;
 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butylboronic acid;
 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-
 35 leucyl]amino]-3-butenylboronic acid;
 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-

valyl]-L-leucyl]amino]-3-butenylboronic acid;

1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanyl]amino]-3-butenylboronic acid;

5 1(R)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]pentylboronic acid;

1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-

10 L-leucyl]amino]propylboronic acid;

1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-L-2-cyclohexylglycyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid; and

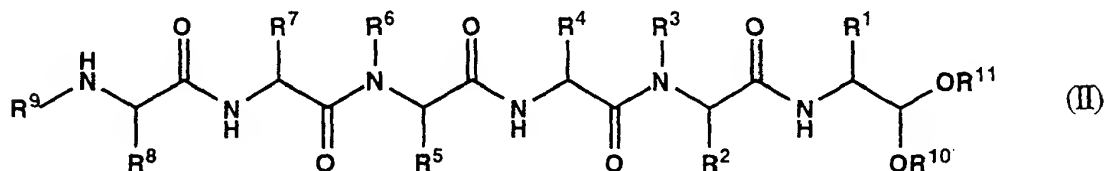
15 1(RS)-[[N-[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl-L-leucyl]-amino]propylboronic acid.

According to the process provided by the present invention, the compounds of formula I hereinbefore and salts of acidic

20 compounds of formula I with bases are manufactured by

a) for the manufacture of a compound of formula I in which E represents CHO, deacetalizing and, where required, deprotecting an acetal of the general formula

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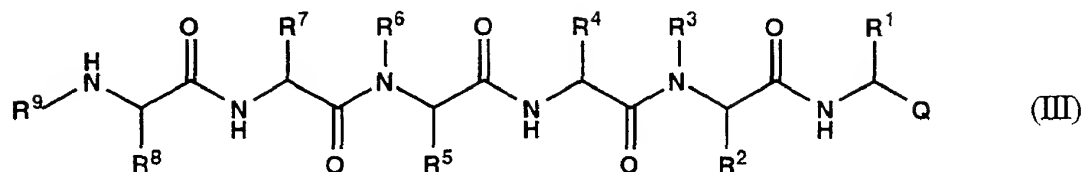
wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ have the significance given earlier, provided that any carboxy, hydroxy and/or aminocarbonyl group(s) present is/are in protected form, and R¹⁰ and R¹¹ each represent lower alkyl,

or

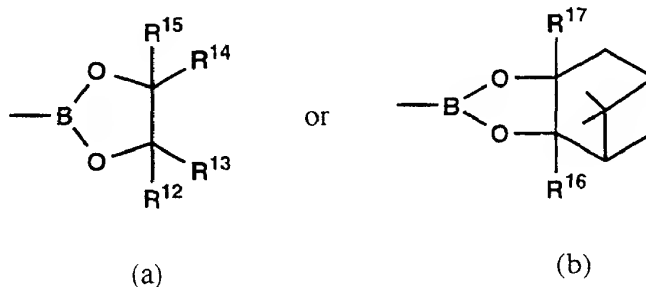
b) for the manufacture of a compound of formula I in which E represents B(OH)₂, ring opening and, where required, deprotecting

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a substituted dioxaborolane of the general formula



- 5 wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ have the significance given earlier, provided that any carboxy, hydroxy and/or aminocarbonyl group(s) present may be in protected form, and Q represents a group of the formula



10

wherein R¹², R¹³, R¹⁴ and R¹⁵ each represent hydrogen or lower alkyl and R¹⁶ and R¹⁷ each represent hydrogen or lower alkyl,

15 and

c) if desired, converting an acidic compound of formula I obtained into a salt with a base.

- 20 Protected carboxy, hydroxy and aminocarbonyl groups which are present in the acetal starting materials of formula II and which may be present in the substituted dioxaborolane starting materials of formula III are carboxy, hydroxy and, respectively, aminocarbonyl groups protected with a conventional protecting
- 25 group known from peptide chemistry. In particular, R², R⁴, R⁷, R⁸ and/or R⁹ can preferably represent tert-butoxycarbonyl-lower alkyl as protected carboxy, R², R⁴, R⁵, R⁷, R⁸ and/or R⁹ can preferably represent lower alkyl O-tert.butyl ether as protected hydroxy and R² can preferably represent tritylaminocarbonyl-
- 30 lower alkyl as protected aminocarbonyl-lower alkyl.

The deacetalization of an acetal of formula II, preferably one in which R^{10} and R^{11} each represent methyl, according to embodiment a) of the process according to the invention can be carried out in a manner known per se. It is conveniently effected using trifluoroacetic acid or an equivalent strong acid in the presence of an inert organic solvent such as a halogenated aliphatic hydrocarbon, e.g. dichloromethane, and in the presence of water. Suitably, the deacetalization is carried out at about room temperature. When protected carboxy, hydroxy and/or aminocarbonyl groups are present in the acetal starting material, these are converted into free carboxy, hydroxy and/or aminocarbonyl groups under the conditions of the deacetalization.

According to a variant of embodiment a) of the process according to the invention, an acetal starting material of formula II is bonded to a solid phase peptide synthesis resin. In this case, cleavage from the resin takes place under the conditions used for the deacetalization.

The ring opening of a substituted dioxaborolane of formula III in which Q represents a group of formula (a), preferably one in which R^{12} , R^{13} , R^{14} and R^{15} each represent methyl, according to embodiment b) of the process according to the invention can also be carried out in a manner known per se. Conveniently, the ring opening is carried out using trifluoroacetic acid or an equivalent strong acid in an inert organic solvent, e.g. a halogenated aliphatic hydrocarbon such as dichloromethane, and optionally in the presence of water. Suitably, the ring opening is carried out at about room temperature. When protected carboxy, hydroxy and/or aminocarbonyl groups are present in the substituted dioxaborolane starting material, these are converted into free carboxy, hydroxy and/or aminocarbonyl groups under the conditions of the ring opening.

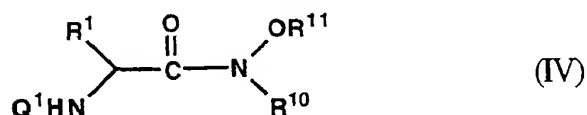
The ring opening of a substituted dioxaborolane of formula III in which Q represents a group of formula (b), especially one in which one of R^{16} and R^{17} represents hydrogen and the other

represents methyl, according to embodiment b) of the process in accordance with the invention can be carried out in a conventional manner. Conveniently, the ring opening is carried out using a periodate, especially an alkali metal periodate, especially sodium
5 periodate in a buffered aqueous-organic medium, suitably at about room temperature. Advantageously, the medium consists of a mixture of an inert water-miscible organic solvent, e.g. acetone, and aqueous ammonium acetate. Any protected carboxy, hydroxy and/or aminocarbonyl group(s) present in the substituted
10 dioxaborolane starting material are deprotected in a manner known per se, e.g. by treatment with trifluoroacetic acid, prior to the ring opening.

According to a variant of embodiment b) of the process
15 according to the invention, a substituted dioxaborolane of formula III in which Q represents a group of formula (a) is bonded to a solid phase synthesis resin. The bonding is typically through an alkyl group R¹², R¹³, R¹⁴ or R¹⁵ linked to the resin via an amide bridge. Cleavage from the resin takes place under the conditions
20 used in embodiment b) of the process.

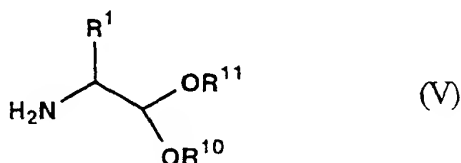
In accordance with embodiment c) of the process acidic compounds of formula I can be converted into salts with bases, e.g. alkali metal salts such as sodium or potassium salts, alkaline
25 earth metal salts such as calcium or magnesium salts, salts with organic bases, e.g. salts with amines such as N-ethylpiperidine, procaine or dibenzylamine, or salts with basic amino acids such as salts with arginine or lysine. The formation and isolation of such salts can be carried out according to methods known per se.
30

The acetal starting materials of formula II are novel and also form an object of the present invention. They can be prepared, for example, by firstly reducing a hydroxamate of the
35 general formula



wherein R^1 , R^{10} and R^{11} have the significance given earlier and Q^1 represents an amino protecting group, e.g. tert.butoxycarbonyl,

with an alkali metal aluminium hydride, e.g. lithium aluminium
5 hydride, treating the product with methanolic hydrochloric acid to give the hydrochloride salt of a compound of the general formula



10

wherein R^1 , R^{10} and R^{11} have the significance given earlier, and subsequently either subjecting this to sequential coupling with respective amino acids or subjecting a fragment obtained during such a sequential coupling to further coupling with a
15 peptide derivative of appropriate length. Alternatively, a compound of formula V can be coupled with a suitable penta-peptide.

The aforementioned coupling reactions can be carried out in
20 a manner known per se in peptide chemistry, conveniently using the respective amino acid or di-, tri-, tetra- or pentapeptide appropriately protected as described above and also at any amino group present by Fmoc [(9-fluorenyl)methoxycarbonyl] in the presence of hydroxybenzotriazole, 1-(3-dimethylaminopropyl)-3-
25 ethylcarbodiimide hydrochloride and N-methylmorpholine and in an inert organic solvent, e.g. a halogenated hydrocarbon such as dichloromethane.

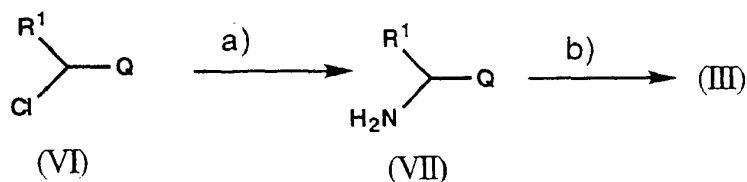
The hydroxamates of formula IV required for the
30 preparation of the acetal starting materials of formula II are known compounds or analogues of known compounds which can be prepared in an analogous manner to the known compounds.

The acetal starting materials of formula II can also be
35 synthesised from a compound of formula V on a solid phase peptide synthesis resin. This procedure is known and is described

in detail in Handbook from Fourth International Symposium on Solid Phase Synthesis and Combinatorial Chemical Libraries, Edinburgh, 1995.

- 5 The substituted dioxaborolanes of formula III used as starting materials in embodiment b) of the process according to the invention are novel and form a further object of the present invention. They can be prepared, for example, as illustrated in Scheme A hereinafter in which R¹ and Q have the significance
10 given earlier:

Scheme A



- Having regard to Scheme A, in step a) a compound of formula
15 VI is reacted with an alkali metal bis[tri(lower alkyl)silyl]amide, e.g. lithium bis(trimethylsilyl)amide, in an inert organic solvent such as an ether, e.g. diethyl ether or tetrahydrofuran, and then treated with a strong acid, e.g. trifluoroacetic acid, to give a compound of formula VII.

20

- In step b) a compound of formula VII is converted into a compound of formula III either by coupling with a pentapeptide, by sequential coupling with respective amino acids or by coupling a fragment obtained during the sequential coupling with a peptide
25 derivative of the desired length, with the amino acid or peptide used being appropriately protected as described above and also at any amino group present by Fmoc. These coupling reactions can be carried out in a manner known per se in peptide chemistry, for example using the amino acid or peptide in the form of a mixed
30 anhydride formed e.g. with a lower alkyl haloformate such as isobutyl chloroformate and carrying out the coupling in the presence of a suitable base, e.g. a tertiary organic base such as N-methylmorpholine.

Substituted dioxaborolanes of formula III obtained by the foregoing coupling and which carry a protecting group on the substituent at R², R⁴, R⁵, R⁷, R⁸ and/or R⁹ can be selectively
5 deprotected in a conventional manner, e.g. using trifluoroacetic acid, to the corresponding compounds which carry a free carboxy, hydroxy and/or aminocarbonyl group on the respective substituent, while retaining the protected boronic acid moiety denoted by Q. These selectively deprotected compounds are also
10 active as inhibitors of proteinases of viral origin and can be used in the treatment of viral infections in the same manner as the compounds of formula I

Compounds of formula VI can be prepared, for example, from
15 a compound of the general formula

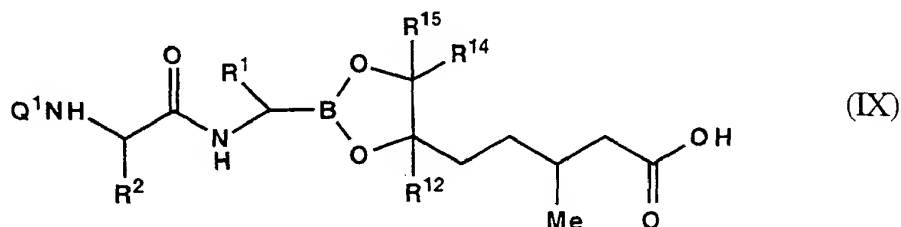


wherein Q has the significance given earlier,
20 which is a known compound or an analogue of a known compound, by reaction with a compound of the formula R^{1a}-MgHal, wherein R^{1a} has the same significance as R¹ hereinbefore, but contains one carbon atom less and Hal represents halogen, preferably bromine. The reaction is carried out under the conventional
25 conditions of a Grignard reaction, for example in an inert organic solvent such as an ether, e.g. diethyl ether or tetrahydrofuran. When Q represents a group of formula (b), the reaction is carried out in the presence of zinc chloride.

30 A compound of formula VI in which R¹ represents bromo-lower alkyl or fluoro-lower alkyl and Q represents a group of formula (a) can be prepared, for example, by hydroborating a bromo- or fluoro-lower alkene, e.g. 3-bromopropene or 3-fluoropropene, reacting the hydroboration product with a diol of
35 the formula R¹²R¹³C(OH)-C(OH)R¹⁴R¹⁵, wherein R¹², R¹³, R¹⁴ and R¹⁵ have the significance given earlier, e.g. 2,3-dimethyl-2,3-butanediol, and reacting the resulting 2-(bromo- or fluoro-lower alkyl)-1,3,2-dioxaborolane with dichloromethane in the presence

of lithium diisopropylamine. The hydroboration can be carried out in a conventional manner, for example using phenylboronic acid at an elevated temperature, e.g. about 100°C, in the absence of a solvent or using borane-dimethyl sulphide complex in the presence of cyclohexene in an inert organic solvent, e.g. dimethoxyethane, at about 0°C followed by treatment with trimethylamine N-oxide.

A substituted dioxaborolane of formula III in which Q represents a group of formula (a) can also be synthesised on a solid phase peptide synthesis resin. For example, a 4-methylbenzhydryl resin can be reacted with a dioxaborolanyl-valeric acid of the general formula



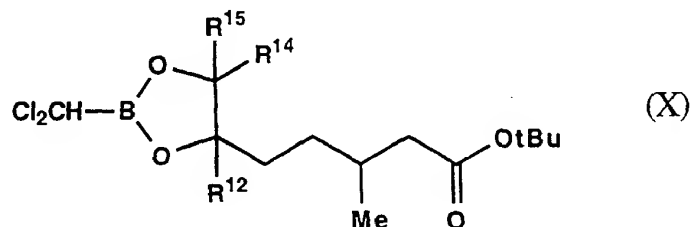
15

wherein R¹, R², R¹², R¹⁴, R¹⁵ and Q¹ have the significance given earlier, and the product can be converted into the required resin-bonded starting material by successive deprotection and coupling with a protected amino acid.

20

Compounds of formula IX can be conveniently prepared by reacting a tert-butyl 6,7-dihydroxy-3,6,7-tri(lower alkyl)-6-octenoate with dichloromethyl diisopropoxyborane, condensing the resulting compound of the general formula

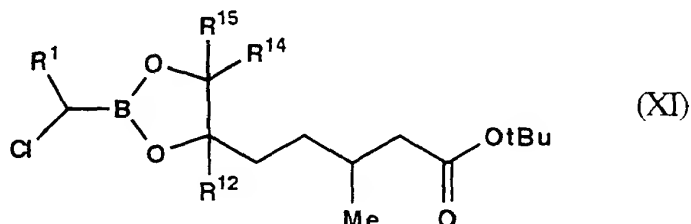
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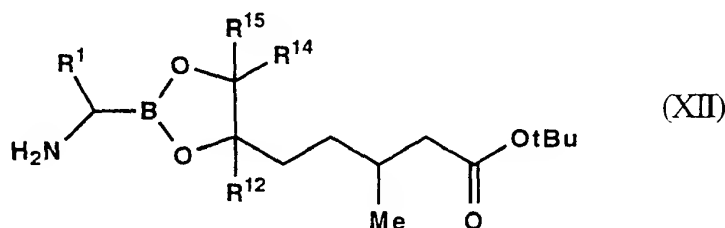
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wherein R¹², R¹⁴ and R¹⁵ have the significance given

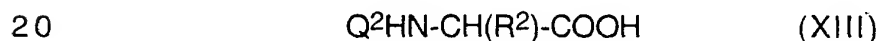
earlier,
 with a compound of formula R^1MgHal , wherein R^1 has the significance given earlier and Hal represents halogen, preferably bromine, under the conditions of a Grignard reaction, reacting the
 5 resulting compound of the general formula



wherein R^1 , R^{12} , R^{14} and R^{15} have the significance given
 10 earlier,
 with an alkali metal bis[tri(lower alkyl)silyl]amide, condensing
 the resulting compound of the general formula

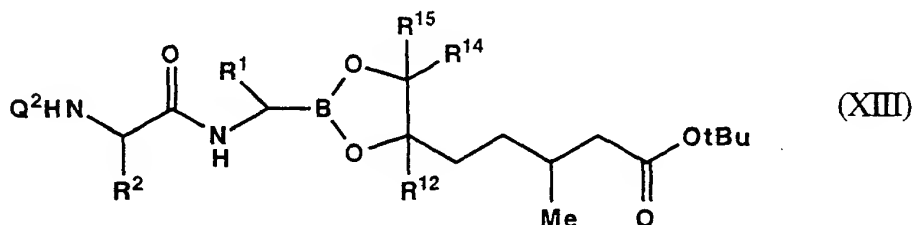


wherein R^1 , R^{12} , R^{14} and R^{15} have the significance given
 15 earlier,
 with a protected amino acid of the general formula



wherein R^2 has the significance given earlier and Q^2
 represents Fmoc,
 and de-esterifying the resulting compound of the general formula

25



wherein R^1 , R^2 , R^{12} , R^{14} , R^{15} and Q^2 have the significance given earlier.

As mentioned earlier, the compounds of formula I and salts of acidic compounds of formula I with bases are inhibitors of proteases of viral origin. The activity against one such protease, namely HCV protease, can be demonstrated using the following assay:

Construction of plasmid for the expression of MBP-NS3''Gly₁₂-NS4A enzyme in E. coli

The nucleotide sequence of this expression plasmid is given in Figure 1 appended hereto and the amino acid sequence of its expression product is given in Figure 2 appended hereto. It is based on the pMAL[®]-c2 vector supplied by New England Biolabs, Inc. (32 Tozer Rd., Beverly, MA, USA). The principle of the construction was to create an in-frame fusion of the maltose binding protein (MBP) gene supplied by the pMAL-c2 vector, and sequences of the HCV genome necessary for NS3 proteinase activity. These HCV sequences were inserted between the EcoRI and HindIII sites of the pMAL-c2 polylinker (positions 2695 and 3556 respectively of the sequence given in Figure 1).

HCV sequences were derived from plasmids pDS 3348-4045 and pBFK 3348-6062, described by Bartenschlager et al, 1993 (Journal of Virology, 67, 3835-3844). Regions encompassing the NS3 proteinase domain (amino acids 1007-1219) and the NS4A domain (amino acids 1658-1711) were isolated and inserted into the pMAL-c2 vector using standard recombinant DNA techniques, including the PCR amplification of required sequences. Between the NS3 and NS4A domains, a linker region was constructed using synthetic oligonucleotides (positions 3343-3390; amino acids 606-621). The resulting plasmid was used to transform E. coli (strain MC1061) cells and expression of the MBP-NS3''Gly₁₂-NS4A enzyme was induced as described below.

Protein expression and purification

E. coli (strain MC1061) cells transformed with the foregoing plasmid were grown in Luria broth containing ampicillin (100 µg/ml) at 37°C. The cells were grown until an optical density of 0.5 at 600 nm had been reached and enzyme expression was then induced by adding 1 mM isopropylthiogalactoside and incubating at 37°C for a further 3 hours. The cells were harvested by centrifugation and stored at -80°C.

10

A pellet from 4 l of bacterial culture was resuspended in E.coli lysis buffer (20 mM Tris HCl, pH 7.5, containing 150 mM NaCl, 1mM EDTA and 10 mM dithiothreitol) and cell lysis was achieved by two passages through a French Pressure cell. The clear supernatant obtained by centrifugation (18000 g, 30 minutes) was then applied to an amylose resin column (4 x 1 cm) (New England Biolabs) which had been equilibrated with ice-cold 50 mM Tris HCl, pH 8.5, containing 200 mM NaCl, 1 mM dithiothreitol and 5% glycerol. The column was washed thoroughly with the equilibration buffer and bound protein was eluted using the equilibration buffer containing 10 mM maltose. Fractions of 1 ml were collected, with fractions containing the enzyme being pooled and stored at -80°C. Enzyme concentration was assayed by the method of M.B. Bradford, Analytical Biochemistry, 1976, vol. 72, p.248.

25

Assay

Compounds of formula I (routinely prepared as stock solutions in DMSO) were assayed for their ability to inhibit the cleavage of a quenched fluorescence substrate [NS4A/B.F peptide (N-[4-[4-(dimethylamino)phenylazo]benzoyl]-L-α-aspartyl-L-α-glutamyl-L-methionyl-L-α-glutamyl-L-α-glutamyl-L-cysteinyl-L-alanyl-L-seryl-L-histidyl-N5-[2-(5-sulpho-1-naphthylamino)-ethyl]-L-glutaminamide); Wilkinson et al, Society for General Microbiology Meeting, University of Warwick, England, 28 March 1996] based on the NS4A/4B cleavage site by enzyme MBP-NS3''Gly 12-NS4A in microtitre plates as follows:

35

The enzyme (1 μ g) was added to 200 μ l final volume of a mixture containing 50 mM Tris HCl, pH 8.5, with 1mM dithiothreitol, 0.1% Triton X-100 and the test compound of formula I. The resulting mixture was incubated at room temperature for 15 minutes prior to starting the reaction by the addition of NS4A/B.F peptide to a final concentration of 10 μ M. The progress of the reaction was evaluated with a Perseptive Biosystems Cytofluor II using an excitation wavelenth of 360 nm and an emission wavelength of 530 nm. After incubation for a further 10 minutes, the reduction in fluorescence in the presence of inhibitor was measured. This was plotted against inhibitor concentration and the inhibitor concentration which caused 50% reduction (IC₅₀) was calculated by manual graphical analysis or by the use of the Perseptive Biosystems Cytocalc curve fitting program.

The results obtained in the foregoing assay with representative compounds of formula I are compiled in the following Table:

Table

| Compound of formula I | HCV proteinase IC ₅₀ (μ mol/l) |
|-----------------------|--|
| A | 0.09 |
| B | 0.07 |
| C | 0.064 |
| D | 0.034 |
| E | 0.038 |
| F | 0.16 |

Compounds:

25

A = 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentenaldehyde.

30

B = 2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4-difluorovaleraldehyde.

- C = 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde.
- 5 D = 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-butenylboronic acid.
- E = 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid.
- 10 F = 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butylboronic acid.

The compounds of formula I and salts of acidic compounds
15 of formula I with bases can be used as medicaments, e.g. in the form of pharmaceutical preparations. The pharmaceutical preparations can be administered enterally such as orally in the form of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions or suspensions, nasally, e.g. in the
20 form of nasal sprays, or rectally, e.g. in the form of suppositories. They may, however, also be administered parenterally, e.g. in the form of injection solutions.

The compounds of formula I and their aforementioned salts
25 can be processed with pharmaceutically inert, organic or inorganic carriers for the production of pharmaceutical preparations. Lactose, corn starch or derivatives thereof, talc, stearic acid or its salts and the like can be used, for example, as such carriers for tablets, coated tablets, dragées and hard
30 gelatine capsules. Suitable carriers for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols and the like; depending on the nature of the active ingredient no carriers are, however, usually required in the case of soft gelatine capsules. Suitable carriers for the production of
35 solutions and syrups are, for example, water, polyols, sucrose, invert sugar, glucose and the like. Suitable carriers for suppositories are, for example, natural or hardened oils, waxes, fats, semi-liquid or liquid polyols and the like.

The pharmaceutical preparations can also contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorants, salts for varying the osmotic pressure, buffers, masking agents or antioxidants. They can also contain still other therapeutically valuable substances.

Medicaments containing a compound of formula I or a salt of an acidic compound of formula I with a base in association with a compatible pharmaceutical carrier are also an object of the present invention, as is a process for the production of such medicaments which comprises bringing one or more of these compounds or salts and, if desired, one or more other therapeutically valuable substances into a galenical administration form together with a compatible pharmaceutical carrier.

As mentioned earlier, the compounds of formula I and salts of acidic compounds of formula I with bases can be used in accordance with the invention as therapeutically active substances, especially as antiviral agents. The dosage can vary within wide limits and will, of course, be fitted to the individual requirements in each particular case. In general, in the case of administration to adults a convenient daily dosage should be about 3 mg to about 3 g, preferably about 10 mg to 1 g. The daily dosage may be administered as a single dose or in divided doses and, in addition, the upper dosage limit referred to earlier may be exceeded when this is found to be indicated.

Finally, the use of compounds of formula I and salts of acidic compounds of formula I with bases for the production of medicaments, especially of antiviral medicaments, is also an object of the invention.

The invention is illustrated by the following Examples. In the Examples SSA denotes the solvent system 0.1% TFA in water and SSB denotes the solvent system 0.1% TFA in 70% acetonitrile 30% water.

Example 1

0.1 g (0.1 mmol) of N²-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(S)-(dimethoxymethyl)propyl]-L-leucinamide was dissolved in
3 ml of dichloromethane, 3 ml of trifluoroacetic acid and 90 mg
of water and the mixture was stirred at room temperature for
30 minutes. The solution was diluted with 20 ml of toluene and
10 the solvent was removed by evaporation. The resulting white
solid was triturated with diethyl ether and filtered off. The solid
was purified by RP-HPLC on a C18 Dynamax column (pore size
300Å; column size 21.4 mm x 50 mm). The elution gradient
comprised 90% SSA 10% SSB to 95% SSB 5% SSA over
15 8.5 minutes. After lyophilization overnight there were obtained
25 mg of 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -
aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-
valyl]-L-leucyl]amino]butyraldehyde as a white foam. MS: m/e
819.5 [M+H]⁺.

20

The starting material was prepared as follows:

i) A solution of 25 g (63.6 mmol) of L-leucine benzyl ester
p-toluenesulphonic acid salt, 14.69 g (63.6 mmol) of N-(tert-
25 butoxycarbonyl)-3-methyl-L-valine, 9.73 g (63.6 mmol) of
1-hydroxybenzotriazole, 7.32 g (63.3 mmol) of N-ethyl-
morpholine and 12.21 g (63.6 mmol) of 1-(3-dimethylamino-
propyl)-3-ethylcarbodiimide hydrochloride in 500 ml of di-
chloromethane was stirred at room temperature overnight. The
30 solution was washed with water, sodium hydrogen carbonate
solution, 2M hydrochloric acid and saturated sodium chloride
solution and dried over anhydrous magnesium sulphate. Evapor-
ation gave 21.65 g of N-[(N-tert-butoxycarbonyl)-3-methyl-L-
valyl]-L-leucine benzyl ester as an oil which was used in the next
35 step without further purification. MS: m/e 435 [M+H]⁺.

ii) A solution of 9.74 g (22.4 mmol) of N-[(N-tert-butoxy-
carbonyl)-3-methyl-L-valyl]-L-leucine benzyl ester in 25 ml of

trifluoroacetic acid and 50 ml of dichloromethane was stirred at room temperature for 30 minutes. The solvent was removed by evaporation and 50 ml of toluene were added. Evaporation gave N-(3-methyl-L-valyl)-L-leucine benzyl ester as an oil which was
5 used in the next step without further purification.

iii) A solution of the foregoing oil, 9 g (22.4 mmol) of N-(9-fluorenylmethoxycarbonyl)-2-methyl-L-phenylalanine, 3.43 g (22.4 mmol) of 1-hydroxybenzotriazole, 3.87 g (33.66 mmol) of
10 N-ethylmorpholine and 4.31 g (22.4 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 100 ml of dichloromethane was stirred at room temperature overnight. The solution was washed with water, sodium hydrogen carbonate solution, 2M hydrochloric acid and saturated sodium chloride
15 solution and dried over anhydrous magnesium sulphate. Evaporation and chromatography on silica gel using 30% ethyl acetate in petroleum ether (b.p. 40-60°C) for the elution gave 12.32 g of N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester as an oil. MS: m/e 718
20 [M+H]⁺.

iv) A solution of 10 g (13.95 mmol) of N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in 30 ml of piperidine and 120 ml of
25 dichloromethane was stirred for 30 minutes at room temperature. The solvent was removed by evaporation and the residue was chromatographed on silica gel using firstly 20% ethyl acetate in hexane and then 10% methanol in dichloromethane for the elution. Evaporation gave 6.9 g of N-[N-[2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in the form of
30 an oil which was used in the next step without further purification.

v) A solution of 6.9 g of the foregoing oil, 2.13 g
35 (13.95 mmol) of 1-hydroxybenzotriazole, 2.68 g (13.95 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 5.93 g (13.95 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-O-tert.-butyl-L- α -glutamic acid in 150 ml of dichloromethane was

stirred at room temperature overnight. The solution was washed with water, sodium hydrogen carbonate solution, 2M hydrochloric acid and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. Evaporation and chromatography of the residue on silica gel using 30% ethyl acetate in petroleum ether (b.p. 40-60°C) for the elution gave 10.89 g of N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester as a thick oil. MS: m/e 903 [M+H]⁺.

10

vi) A solution of 10.89 g (12.07 mmol) of N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in 30 ml of piperidine and 120 ml of dichloromethane was stirred for 30 minutes at room temperature. The solvent was removed by evaporation and the residue was chromatographed on silica gel using firstly 20% ethyl acetate in hexane and then 10% methanol in dichloromethane for the elution. Evaporation gave N-[N-[N-[O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in the form of an oil which was used in the next step without further purification.

vii) A solution of the foregoing oil, 4.96 g (12.07 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -aspartic acid, 1.85 g (12.07 mmol) of 1-hydroxybenzotriazole and 2.32 g (12.07 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 100 ml of dichloromethane was stirred at room temperature overnight. The solution was washed with water, sodium hydrogen carbonate solution, 2M hydrochloric acid and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. Evaporation and chromatography of the residue on silica gel using ethyl acetate for the elution gave 10.088 g of N-[N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester as a white solid. MS: m/e 1074 [M+H]⁺.

viii) A solution of 10.088 g (9.4 mmol) of N-[N-[N-[N-[(9-fluorenyl)methoxycarbonyl] O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in 30 ml of piperidine and 120 ml of dichloromethane was stirred for 30 minutes at room temperature. The solvent was removed by evaporation and the residue was chromatographed on silica gel using firstly 20% ethyl acetate in hexane and then 10% methanol in dichloromethane for the elution. Evaporation gave N-[N-[N-[O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl-L-leucine benzyl ester in the form of an oil which was used in the next step without further purification.

ix) A solution of 8 g of the foregoing oil, 1.64 g (9.4 mmol) of tert-butyl hydrogen succinate, 1.44 g (9.4 mmol) of 1-hydroxy-benzotriazole and 1.805 g (9.4 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride in dichloromethane was stirred at room temperature overnight. The solution was washed with water, sodium hydrogen carbonate solution, 2M hydrochloric acid and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. Evaporation and trituration of the residue with acetone gave 6.87 g of N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester as a white solid. MS: m/e 1008.6 [M+H]⁺, m/e 1030.3 [M+Na]⁺.

x) A solution of 6.8 g (6.75 mmol) of N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in 200 ml of dimethylformamide was hydrogenated over 600 mg of 10% palladium/carbon for 1 hour. The catalyst was removed by filtration and the filtrate was evaporated to give 15 g of crude product which was chromatographed on silica gel using 10-15% methanol in dichloromethane for the elution to give 6 g of N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-

valyl]-L-leucine as a white solid of melting point 235-236°C; MS: m/e 918.4 [M+H]⁺, m/e 940.3 [M+Na]⁺.

- xi) 370 mg (2.5 mmol) of N,O-dimethyl 2(S)-(tert-butoxy-formamido)butyrohydroxamate were dissolved in 20 ml of anhydrous tetrahydrofuran under nitrogen and the solution was cooled to 0°C in an ice-bath. 1.5 ml (1.5 mmol) of 1M lithium aluminium hydride in tetrahydrofuran were added and the mixture was stirred at 0°C for 10 minutes. 20 ml of saturated aqueous potassium hydrogen sulphate were added and the mixture was stirred vigorously under nitrogen for 30 minutes at room temperature. The mixture was then diluted with 50 ml of diethyl ether and the organic layer was separated, dried over anhydrous magnesium sulphate and the solvent was evaporated. The residue was dissolved in 10 ml of a saturated methanolic hydrogen chloride solution, stirred for 1 hour, then diluted with 50 ml of toluene and evaporated to dryness. The resulting oil was dissolved in 10 ml of dichloromethane and 184 mg (0.2 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 58 mg (0.3 mmol) of 2-(3-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride, 41 mg (0.3 mmol) of 1-hydroxy-7-azabenzotriazole and 350 mg (3.0 mmol) of N-ethylmorpholine were added. The mixture was stirred for 30 minutes then washed in sequence with saturated sodium bicarbonate solution and 2M hydrochloric acid and dried over anhydrous magnesium sulphate. The solution was evaporated to dryness and the residue was chromatographed on silica gel using 4% methanol in dichloromethane for the elution. After trituration with diethyl ether there were obtained 110 mg of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)propyl]-leucinamide as a white solid of melting point 242-244°C. MS: m/e 1001.5 [M+H-MeOH]⁺, m/e 1055 [M+Na]⁺.

Analysis for C₅₃H₈₈O₁₄N₆ [1033.315].

Calculated: C, 61.61; H, 8.58; N, 8.13%

Found: C, 61.52, H, 8.45; N, 8.19%

Example 2

70 mg (0.067 mmol) of N²-[N-[N-[N-[3-(tert-butoxy-
5 carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide were stirred
in a mixture of 4 ml of trifluoroacetic acid, 4 ml of dichloro-
methane and 30 mg of water at room temperature for 30 minutes.
10 The solution was evaporated to dryness in a vacuum and the
residue was chromatographed on silica gel using dichloro-
methane/methanol/acetic acid/water (60:13:2:2) for the elution.
There were obtained 36 mg of 2(RS)-[[N-[N-[N-[N-[N-[(3-carboxy-
propionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenyl-
15 alanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentynal (9:1
mixture of diastereoisomers) as a white solid. MS: m/e 829.6
[M+H]⁺.

The starting material was prepared as follows:

20

i) A solution of 12.17 g (57.14 mmol) of N-(tert-butoxy-
carbonyl)-1(S)-amino-4-pentynoic acid, 8.74 g (64.74 mmol) of
hydroxybenzotriazole, 6.96 g (71.43 mmol) of N,O-dimethyl-
hydroxylamine, 8.21 g (71.43 mmol) of N-ethylmorpholine and
25 13.7 g (71.43 mmol) of 1-(3-dimethylaminopropyl)-3-ethyl-
carbodiimide hydrochloride in 250 ml of dichloromethane was
stirred for 18 hours, then washed with 2M hydrochloric acid and
saturated sodium bicarbonate solution, dried and evaporated to
give 14.2 g of N,O-dimethyl 2(S)-(tert-butoxyformamido)-4-
30 pentynohydroxamate as a viscous gum which slowly crystallized.
Analysis for C₁₂H₂₀N₂O₄ [256.302].
Calculated: C, 56.24; H, 7.87; N, 10.93%
Found: C, 56.01, H, 7.81; N, 10.92%

35 ii) 10 ml (10 mmol) of 1M lithium aluminium hydride in tetra-
hydrofuran were added to a solution of 3.15 g (12.3 mmol) of
N,O-dimethyl 2(S)-(tert-butoxyformamido)-4-pentynohydrox-
amate in 50 ml of dry tetrahydrofuran at 0°C under a nitrogen

atmosphere. The solution was stirred for 20 minutes and then 40 ml of saturated potassium hydrogen sulphate solution were added dropwise. The mixture was stirred for 15 minutes and then diluted with diethyl ether. The organic layer was dried over magnesium sulphate and evaporated to give an oil which was dissolved in 50 ml of methanolic hydrogen chloride solution. The solution was left at room temperature for 1 hour and then evaporated to dryness to give a dark brown gum. 1.05 g of the gum were added to a solution of 2.06 g (5.84 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-L-leucine, 867 mg (6.42 mmol) of hydroxybenzotriazole, 1.233 g (6.42 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 2.216 g (19.27 mmol) of N-ethylmorpholine in 40 ml of dichloromethane. The solution was stirred at room temperature for 18 hours, washed with 2M hydrochloric acid and saturated sodium bicarbonate solution, dried over magnesium sulphate and evaporated to give a gum which was chromatographed on silica gel using ethyl acetate/petrol (2:3) for the elution. There were obtained 1.1 g of N2-[(9-fluorenyl)methoxycarbonyl]-N1-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide as an off-white solid. ¹H NMR (400 MHz, DMSO-d₆) δ: 0.86 (6H,dd), 1.35-1.65 (3H,m), 2.22-2.39 (2H,m), 2.75(1H,t), 3.22 (3H,s), 3.27 (3H,s), 3.91 (1H,m), 4.08 (1H,m), 4.15-4.3 (4H,m), 7.29 (2H,m), 7.4 (2H,t), 7.42 (1H,d), 7.71 (2H,d), 7.84 (1H,d), 7.88 (2H,d).

iii) 525 mg (1.1 mmol) of N2-[(9-fluorenyl)methoxycarbonyl]-N1-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide were dissolved in 20 ml of dichloromethane and 5 ml of piperidine and the mixture was stirred at room temperature for 30 minutes. The mixture was evaporated to dryness and the residue was chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. Evaporation of the dichloromethane solution gave a gum which was added to a solution of 363 mg (1.03 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-leucine, 149 mg (1.1 mmol) of hydroxybenzotriazole and 288 mg (1.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 15 ml of dichloromethane. The mixture was stirred for

18 hours, then washed with 2M hydrochloric acid and saturated sodium bicarbonate solution, dried over magnesium sulphate and evaporated to dryness. The residue was chromatographed on silica gel using ethyl acetate/petrol (1:2) for the elution to give
5 501 mg of N₂-[N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valyl]-N₁-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide as a white foam. MS: m/e 592.3 [M+H]⁺, 560.3 [M+H-MeOH]⁺.

iv) 490 mg (0.83 mmol) of N₂-[N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valyl]-N₁-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide were dissolved in 16 ml of dichloromethane and 4 ml of piperidine and the mixture was stirred at room temperature for 30 minutes. The mixture was evaporated to dryness and the residue was chromatographed on silica gel
15 using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. Evaporation of the dichloromethane solution gave a gum which was added to a solution of 321 mg (0.8 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanine, 122 mg (0.9 mmol) of
20 hydroxybenzotriazole and 192 mg (1 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 15 ml of dichloromethane. The mixture was stirred for 18 hours, then washed with 2M hydrochloric acid and saturated sodium bicarbonate solution, dried over magnesium sulphate and
25 evaporated to dryness. The residue was chromatographed on silica gel using ethyl acetate/petrol (2:3) for the elution to give a white foam which was dissolved in 16 ml of dichloromethane and 4 ml of piperidine and left at room temperature for 30 minutes. The mixture was evaporated to dryness and the residue was
30 chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. Evaporation of the dichloromethane solution gave a gum which was added to a solution of 213 mg (0.5 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamic acid,
35 74 mg (0.55 mmol) of hydroxybenzotriazole and 115 mg (0.6 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 10 ml of dichloromethane. The mixture was stirred for 18 hours, then washed with 2M hydrochloric acid and

saturated sodium bicarbonate, dried over magnesium sulphate and evaporated to dryness. Trituration of the residue with diethyl ether gave 345 mg of N²-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)-methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-butynyl]-leucinamide as a white solid. MS: m/e 938 [M+H]⁺, 906 [M+H-MeOH]⁺.

v) 340 mg (0.36 mmol) of N²-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide were dissolved in 12 ml of dichloromethane and 3 ml of piperidine and the mixture was stirred at room temperature for 30 minutes. The mixture was evaporated to dryness and the residue was chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. Evaporation of the dichloromethane solution gave a gum which was added to a solution of 144 mg (0.35 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -aspartic acid, 54 mg (0.4 mmol) of hydroxybenzotriazole and 96 mg (0.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 15 ml of dichloromethane. The mixture was stirred for 18 hours, then washed with 2M hydrochloric acid and saturated sodium bicarbonate solution, dried over magnesium sulphate and evaporated to dryness. Trituration of the residue with diethyl ether gave 360 mg of N²-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide as a white solid. MS: m/e 1077 [M+H-MeOH]⁺.

vi) 350 mg (0.32 mmol) of N²-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-butynyl]-L-leucinamide were dissolved in 12 ml of dichloromethane and 3 ml of piperidine and the mixture was stirred at room temperature for 30 minutes.

The mixture was evaporated to dryness and the residue was chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. Evaporation of the dichloromethane solution gave a foam which
5 was added to a solution of 104 mg (0.6 mmol) of succinic acid monotert-butyl ester, 81 mg (0.6 mmol) of hydroxybenzotriazole and 192 mg (1 mmol) of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride in 10 ml of dichloromethane. The mixture was stirred for 18 hours, then washed with 2M hydro-
10 chloric acid and saturated sodium bicarbonate solution, dried over magnesium sulphate and evaporated to dryness. Chromatography of the residue on silica gel using 4% methanol in dichloromethane for the elution and trituration with ethyl acetate gave 145 mg of
15 N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-3-butyryl]-L-leucinamide as a white solid. MS: m/e 1043 [M+H]⁺, 1011 [M+H-MeOH]⁺.

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Example 3

94 mg (0.86 mmol) of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)-propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-
25 [3,3,3-trifluoro-1(S)-(dimethoxymethyl)propyl]-L-leucinamide were stirred in a mixture of 4 ml of trifluoroacetic acid, 4 ml of dichloromethane and 30 mg of water at room temperature for 30 minutes. The solution was evaporated to dryness in a vacuum and the residue was chromatographed on silica gel using dichloro-
30 methane/methanol/acetic acid/water (120:15:3:2) for the elution. There were obtained 41 mg of 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde (7:1 mixture of diastereoisomers) as a white solid. MS:
35 m/e 873 [M+H]⁺.

The starting material was prepared as follows:

184 mg (0.2 mmol) of N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine were suspended in 6 ml of dichloromethane and treated
5 with 34 mg (0.25 mmol) of hydroxybenzotriazole followed by 391 mg (1.75 mmol) of 3,3,3-trifluoro-1(S)-dimethoxymethylpropylamine hydrochloride and 690 mg (6 mmol) of N-ethylmorpholine. The mixture was stirred for 2 hours, then washed in sequence with 2M hydrochloric acid and saturated sodium
10 bicarbonate solution and dried over magnesium sulphate. The solvent was removed by evaporation and the resulting solid, after trituration with diethyl ether, was chromatographed on silica gel using 4% methanol in dichloromethane for the elution. There were obtained 101 mg of N²-[N-[N-[N-[N-[(3-tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[3,3,3-trifluoro-1(S)-
15 (dimethoxymethyl)propyl]-L-leucinamide as a white solid. MS: m/e 1088 [M+H]⁺.

20

Example 4

0.02 g (0.006 mmol) of 5-[4-[[N-[N-[N-[(9-fluorenyl)-methoxycarbonyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]-N-3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]-amino]methyl]-3,5-dimethoxyphenoxy]-N-(4-methyl- α -(RS)-phenylbenzyl)valeramide-polystyrene conjugate was suspended
25 and agitated in 0.7 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and then resuspended in and agitated with 0.7 ml of dimethylformamide/piperidine (4:1)
30 for a further 5 minutes. The resin was then drained and washed five times with 1.5 ml of dimethylformamide.

The resin was then suspended in a solution of 0.026 g (0.06 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine in 0.3 ml of dimethylformamide and then a
35 mixture of 0.019 g (0.06 mmol) of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.012 g (0.12 mmol) of N-methylmorpholine dissolved in 0.3 ml of

dimethylformamide was added. After agitating for 2 hours the resin was drained and washed five times with 1.5 ml of dimethylformamide.

- 5 The resin was resuspended in and agitated with 1.5 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethylformamide/piperidine(4:1) for a further 5 minutes. Then, the resin was drained and washed five times with 1.5 ml of
10 dimethylformamide.

- The resin was then suspended in a solution of 0.025 g (0.06 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -aspartic acid in 0.3 ml of dimethylformamide and then a
15 mixture of 0.019 g (0.06 mmol) of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoraborate and 0.012 g (0.12 mmol) of N-methylmorpholine dissolved in 0.3 ml of dimethylformamide was added. After agitating for 2 hours the resin was drained and washed five times with 1.5 ml of
20 dimethylformamide.

- The resin was resuspended in and agitated with 1.5 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethylformamide/piperidine (4:1) for a further 5 minutes. Then, the
25 resin was drained and washed five times with 1.5 ml of dimethylformamide.

- The resin was then suspended in a solution of 0.01 g
30 (0.06 mmol) tert-butyl hydrogen succinate in 0.3 ml of dimethylformamide and treated with a mixture of 0.019 g (0.06 mmol) 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.012 g (0.12 mmol) of N-methylmorpholine dissolved in 0.3 ml of dimethylformamide. After
35 agitating for 2 hours the resin was drained and washed 5 times with 1.5 ml of dimethylformamide and then twice with 1.5 ml of dichloromethane.

The resin was treated with 0.8 ml of trifluoroacetic acid/water (19:1) and then agitated for 30 minutes. It was then filtered off and washed with 0.8 ml of trifluoroacetic acid/water (19:1). The combined trifluoroacetic acid/water mixtures
5 were then evaporated in a vacuum centrifuge and the residue was suspended in 0.8 ml of acetonitrile/water (1:1) and freeze dried. There were obtained 6.3 mg of 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-3-(2-naphthyl)-D-alanyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-tri-
10 fluorobutyraldehyde as a white solid; MS: m/e 941.5 [M+H]⁺.

The starting material was prepared as follows:

i) 18 g (60.0 mmol) of N,O-dimethyl 2(RS)-(tert-butoxy-
15 formamido)-4,4,4-trifluorobutyrohydroxamate were dissolved in 230 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C. 48 ml (48 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran were then added dropwise while maintaining the temperature at 0°C. The mixture was stirred for
20 10 minutes at 0°C and then the reaction was quenched by the dropwise addition of saturated potassium hydrogen sulphate solution to pH 1 while maintaining the temperature at below 20°C. The resulting white slurry was stirred vigorously for a further 30 minutes and was then partitioned in three equal
25 aliquots of diethyl ether. The combined diethyl ether fractions were washed with saturated sodium chloride solution, dried over anhydrous magnesium sulphate, filtered and evaporated. The residue was then dissolved in 100 ml of anhydrous saturated methanolic hydrogen chloride solution and left overnight at 4°C.
30 The mixture was evaporated and the residue was triturated with dichloromethane. The filtrate was evaporated and the residue was chromatographed on silica gel using 5% methanol, 3% acetic acid and 1.5% water in dichloromethane for the elution. There were obtained 8.80 g of 3,3,3-trifluoro-2(RS)-(dimethoxy-
35 methyl)-propylamine hydrochloride as a white solid. ¹H NMR : (CDCl₃) δ : 2.60-2.96 (m,2H), 3.49 (d,6H), 3.57-3.69 (q,1H), 4.66 (d,1H), 8.72 (br s,3H).

- ii) To a stirred mixture of 5.6 g (25.0 mmol) of 3,3,3-trifluoro-2(RS)-(dimethoxymethyl)-propylamine hydrochloride 3.65 ml of triethylamine, 7.8 g (25.0 mmol) of 4-[4-(ethoxycarbonyl)butoxy]2,6-dimethoxybenzaldehyde and 25 g of 3Å molecular sieves in dichloromethane were added 5.8 g (27.5 mmol) of sodium triacetoxyborohydride. After 3 hours the molecular sieves were removed by filtration. The filtrate was then washed with three equal aliquots of saturated sodium bicarbonate solution and dried over anhydrous magnesium sulphate and filtered. The solvent was removed by evaporation and the resulting orange oil was chromatographed on silica gel using 60% ethyl acetate in hexane for the elution. There were obtained 10.4 g of ethyl 5-[4-[[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propylamino]methyl]-3,5-dimethoxyphenoxy]valerate as a pale orange oil; ^1H NMR : (CDCl_3) δ : 1.25 (t,3H), 1.78-1.87 (m,4H), 2.18-2.52 (m,4H), 2.86-2.92 (m,1H), 3.33 (d,6H), 3.77 (s,6H), 3.81 (d,2H), 3.96 (t,2H), 4.13 (q,2H), 4.26 (d,1H), 6.18 (s,2H); MS: m/e 482.2 [M+H], 504.2 [M+Na].
- iii) A solution of 6.6 g (18.7 mmol) of N-[(9-fluorenyl)-methoxycarbonyl]-L-leucine and 9.7 g (18.7 mmol) of 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate in 50 ml of anhydrous dichloromethane was stirred at room temperature for 15 minutes. To this mixture were then added 6.0 g (12.4 mmol) of ethyl 5-[4-[[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propylamino]methyl]-3,5-dimethoxyphenoxy]valerate and 4.3 ml of (24.8 mmol) diisopropylethylamine. After stirring overnight at 25°C the mixture was diluted with dichloromethane and washed in sequence with water, 10% citric acid solution, saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, then dried over anhydrous magnesium sulphate and filtered. The solvent was removed by evaporation and the residue was chromatographed on silica gel using 30% ethyl acetate in hexane for the elution. There were obtained 8.06 g of ethyl 5-[4-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]amino]methyl]-3,5-dimethoxyphenoxy]valerate; MS: m/e 839.4 [M+Na], 855.3 [M+K].

iv) 8.0 g (9.8 mmol) of 5-[4-[[N-[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]amino]methyl]-3,5-dimethoxyphenoxy]valerate and 40 ml
5 of piperidine were dissolved in 145 ml of dry dichloromethane and the solution was stirred at room temperature for 30 minutes. It was then evaporated in a vacuum and the residue was chromatographed on silica gel using 2% methanol, 49% dichloromethane and 49% hexane followed by 5% methanol, 47.5% dichloromethane
10 and 47.5% hexane for the elution. There were obtained 4.09 g of ethyl 5-[4-[[N-[3,3,3-trifluoro-1(RS)-dimethoxymethyl]propyl]-N-(L-leucyl)amino]methyl]-3,5-dimethoxyphenoxy]valerate as a clear stiff oil; MS: m/e 595 [M+H].

15 v) A solution of 2.76 g (7.8 mmol) of N-[(9-fluorenyl)-methoxycarbonyl]-3-methyl-L-valine, 1.60 g (8.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1.60 g (10.7 mmol) of N-hydroxybenzotriazole in 70 ml of dichloromethane was stirred at 0°C for 15 minutes. There were
20 then added 4.06 g (7.1 mmol) of ethyl 5-[4-[[N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]-N-(L-leucyl)-amino]methyl]-3,5-dimethoxyphenoxy]valerate and 2.7 ml (21.3 mmol) of N-ethyl-morpholine in 70 ml of dichloromethane. After stirring overnight at room temperature the mixture was washed in sequence with
25 10% citric acid solution, saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, dried over anhydrous magnesium sulphate, filtered and evaporated. The residue was chromatographed on silica gel using 35% ethyl acetate in hexane for the elution. There were obtained 6.11 g of
30 ethyl 5-[4-[[N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-(dimethoxyethyl)propyl]amino]methyl]-3,5-dimethoxy-phenoxy]valerate as a white foam; MS: m/e 952.5 [M+Na], 968.5 [M+K].

35 vi) 5.8 g (6.3 mmol) of ethyl 5-[4-[[N-[N-[N-[(9-fluorenyl)-methoxycarbonyl]-3-methyl-L-valyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-(dimethoxyethyl)propyl]amino]methyl]-3,5-dimethoxy-phenoxy]valerate and 18 ml of piperidine were

- dissolved in 90 ml of dichloromethane and the solution was stirred at room temperature for 1 hour. It was then evaporated and the residue was chromatographed on silica gel using 3% methanol, 48.5% dichloromethane and 48.5% hexane for the
- 5 elution. There were obtained 4.1 g of ethyl 5-[4-[[N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]-N-[N-(3-methyl-L-valyl)-L-leucyl]amino]methyl]-3,5-dimethoxyphenoxy]-valerate as a white foam; MS: m/e 708.6 [M+H], 730.5 [M+Na].
- 10 vii) 4.0 g (5.7 mmol) of ethyl 5-[4-[[N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]-N-[N-(3-methyl-L-valyl)-L-leucyl]-amino]methyl]-3,5-dimethoxyphenoxy]-valerate were dissolved in 40 ml of methanol. 2.4 g (17.3 mmol) of potassium carbonate and 8.0 ml of water were then added and the mixture was stirred
- 15 for 2 days at room temperature. The solvent was removed by evaporation and the residue was dissolved in 20 ml of water and 20 ml of dioxan. 2.9 g (8.6 mmol) of N-[(9-fluorenyl)-methoxycarbonyloxy]-succinimide were then added and the mixture was stirred for 3 hours. The mixture was adjusted to pH 3 with 10%
- 20 citric acid and then washed with three equal aliquots of dichloromethane. The combined organic layers were washed with saturated sodium chloride solution, dried over anhydrous magnesium sulphate, filtered and the filtrate was evaporated. The residue was chromatographed on silica gel using 4% tert-
- 25 butyl methyl ether in dichloromethane for the elution. There were obtained 5.12 g of 5-[4-[[N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-(dimethoxymethyl)propyl]amino]methyl]-3,5-dimethoxyphenoxy]-valeric acid as a white foam; MS: m/e 870.8 [M+H-MeOH], 888.7
- 30 [M+H-CH₃], 889.7 [M-CH₃] 902.7 [M+H], 924.7 [M+Na].

- viii) 5.4 g (5.4 mmol) of 4-methylbenzhydrylamine resin were swollen in 30 ml of dimethylformamide, excess solvent was drained from the resin and it was then washed twice with 20 ml
- 35 dimethylformamide/N-methylmorpholine (9:1). The resin was then resuspended in 10 ml of dimethylformamide containing 4.98 g (5.4 mmol) of 5-[4-[[N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valyl]-L-leucyl]-N-[3,3,3-trifluoro-1(RS)-

- dimethoxymethyl)propyl]amino]methyl-3,5-dimethoxyphenoxy]-
valeric acid and 1.74 g (5.4 mmol) of 2-(1H-benzotriazol-1-yl)-
1,1,3,3-tetramethyluronium tetrafluoraborate. Thereto there
were added 1.18 ml (10.8 mmol) of N-methylmorpholine
5 dissolved in 10 ml of dimethylformamide. The resulting mixture
was agitated for 2 hours and the resin was then drained and
washed five times with 30 ml of dimethylformamide. The resin
was then resuspended in 30 ml of dimethylformamide containing
2.03 ml (21.6 mmol) of acetic anhydride and 2.96 ml (27 mmol)
10 of N-methylmorpholine. This mixture was agitated for
30 minutes and the resin was then drained and washed five times
with 30 ml of dimethylformamide each time. The resin was
resuspended in and agitated in 30 ml of dimethylformamide/
piperidine (4:1). After 5 minutes the resin was drained,
15 resuspended and again agitated in the foregoing dimethylform-
amide/piperidine mixture for a further 5 minutes. The resin was
then drained and washed five times with 30 ml of dimethyl-
formamide.
- 20 ix) A solution of 3.2 g (8.1 mmol) of N-[(9-fluorenyl)methoxy-
carbonyl]-3-(2-methylphenyl)-L-alanine and 2.17 g (6.75 mmol)
of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetra-
fluoroborate in 22 ml of dimethylformamide was added to the
resin from paragraph viii) and subsequently 1.5 ml (13.5 mmol)
25 of N-methylmorpholine were added. The mixture was agitated for
30 minutes and then the resin was drained and washed five times
with 30 ml of dimethylformamide, twice with 30 ml of
dichloromethane, twice with 30 ml of ethyl acetate and twice
with 30 ml of diethyl ether. After drying there were obtained
30 8.95 g of 5-[4-[[N-[N-[N-[(9-fluorenyl)methoxycarbonyl]-2-
methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]-N-[3,3,3-
trifluoro-1(RS)-(dimethoxymethyl)propyl]amino]methyl]-3,5-
dimethoxyphenoxy]-N-(4-methyl- α -(RS)-phenylbenzyl)-
valeramide-polystyrene conjugate as a pale brown solid
35 (0.31 mmol/g loading estimated by quantitation of dibenzo-
fulvene at 301 nm).

Example 5

0.236 g (0.215 mmol) of N²-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide was dissolved in 1.5 ml of water,
13.5 ml of trifluoroacetic acid and 7 ml of dichloromethane and the solution was stirred at room temperature for 1 hour and then
10 left to stand at 4°C for 18 hours. The solution was then diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off. The solid was purified by RP-HPLC on a Dynamax C18 column (5 micron, 300Å, 21.4 mm x 50 mm). The elution gradient comprised 95%
15 SSA:5% SSB to 95%:SSB 5% SSA over 6 minutes and there were obtained, after lyophilization, 69 mg of 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-butenylboronic acid as a foam; MS: m/e 847 [M+H].

20

The starting material was prepared as follows:

i) 2 g (9.48 mmol) of 2-(dichloromethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane were dissolved in 30 ml of tetra-
25 hydrofuran and the solution was cooled under a nitrogen atmosphere to -78°C. 9.5 ml (9.5 mmol) of 1M allylmagnesium bromide were added dropwise and the solution was stirred at room temperature for 18 hours. The solution was partitioned between ethyl acetate, saturated sodium chloride solution and 2M hydro-
30 chloric acid solution. The aqueous layer was extracted with ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulphate. After filtration and evaporation the oil obtained was distilled to give 1.45 g of 2-(1(RS)-chloro-3-butenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane; b.p.
35 53°C/0.4 mm Hg.

ii) 6.6 ml (6.6 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran were added dropwise to a solution of 1.43 g

(6.6 mmol) of 2-(1(RS)-chloro-3-butenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane in 20 ml of tetrahydrofuran under nitrogen at -78°C. The solution was then stirred overnight at room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration and the filtrate was cooled to 0°C. 1.5 ml (19.8 mmol) of trifluoroacetic acid were added and the solution was stirred at 0°C for 30 minutes. The resulting precipitate was filtered off and dried to give 0.5 g of α -(RS)-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate which was used in the next step without further purification.

iii) 0.25 g (0.27 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 4 ml of dimethylformamide and 4 ml of dichloromethane. 0.15 ml (1.6 mmol) of N-methylmorpholine was added and the solution was cooled to -15°C under a nitrogen atmosphere. 50 mg (0.38 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -15°C. 0.1 g (0.32 mmol) of α -(RS)-allyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate was added and the mixture was stirred at room temperature for 18 hours. After evaporation the residue was partitioned between ethyl acetate and 2M hydrochloric acid. The organic layer was washed with 2M hydrochloric acid, water and saturated sodium chloride solution and then dried over anhydrous sodium sulphate. After evaporation there was obtained 0.3 g of N₂-N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N₁-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide in the form of a solid; MS: m/e 1097 [M+H].

35

Example 6

0.25 g (0.23 mmol) of N²-N-[N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]-L-leucinamide was dissolved in 1.5 ml of water, 13.5 ml of trifluoroacetic acid and 7 ml of dichloromethane and the solution was stirred at room temperature for 1 hour and then left to
10 stand at 4°C for 18 hours. The solution was diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off. The solid was purified by RP-HPLC on an Aquapore octyl column (20 micron, 100 mm x 10 mm). The elution gradient comprised 95% SSA:5% SSB to 5%
15 SSA:95% SSB over 6 minutes and there were obtained, after lyophilization, 92 mg of 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid as a foam; MS: m/e 835 [M+H].

20

The starting material was prepared as follows:

i) 2.64 g (12.5 mmol) of 2-(dichloromethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane were dissolved in 30 ml of
25 tetrahydrofuran and the solution was cooled under a nitrogen atmosphere to -78°C. 11.8 ml (12.5 mmol) of 1.06M ethylmagnesium bromide were added dropwise and the solution was stirred at room temperature for 18 hours. The solution was partitioned between ethyl acetate, saturated sodium chloride
30 solution and 2M hydrochloric acid solution. The aqueous layer was extracted with ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulphate. After filtration and evaporation the oil obtained was distilled to give 2.04 g of 2-[1(RS)-chloropropyl]-4,4,5,5-tetramethyl-1,3,2-
35 dioxaborolane; b.p. 53°C/0.8 mm Hg.

ii) 10 ml (10 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran were added dropwise to a solution of 2.03 g

(9.9 mmol) of 2-[1(RS)-chloropropyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane in 20 ml tetrahydrofuran under a nitrogen atmosphere at -78°C. The solution was then stirred overnight at room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration and the filtrate was cooled to 0°C. 2.3 ml (30 mmol) of trifluoroacetic acid were added and the solution was stirred at 0°C for 30 minutes. The resulting precipitate was filtered off and dried to give 0.5 g of α -(RS)-ethyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate as a white solid.

Analysis for $C_{11}H_{21}BNF_3O_4$ [299.15].

Calculated: C, 44.17; H, 7.08; N, 4.68%

Found: C, 44.06, H, 7.05, N, 4.71%.

iii) 0.25 g (0.27 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 5 ml of dichloromethane. 0.15 ml (1.6 mmol) of N-methylmorpholine was added and the solution was cooled to -15°C under a nitrogen atmosphere. 50 mg (0.38 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -15°C. 0.1 g (0.33 mmol) of α -(RS)-ethyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate was added and the mixture was stirred at room temperature for 18 hours. After evaporation the residue was partitioned between ethyl acetate and 2M hydrochloric acid. The organic layer was washed with 2M hydrochloric acid, water and saturated sodium chloride solution and then dried over anhydrous sodium sulphate. After evaporation there was obtained 0.26 g of N₂-N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N₁-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)propyl]-L-leucinamide in the form of a solid; MS: m/e 1085 [M+H].

Example 7

0.16 g (14.6 mmol) of N²-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide was dissolved in 4 ml of trifluoroacetate acid and 4 ml of dichloromethane. 4 drops of water were added and the solution was stirred at room temperature for 3 hours. The
10 residue was triturated with diethyl ether and the resulting solid was filtered off and dried to give, after lyophilization, 139 mg of 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butylboronic acid as a foam; MS: m/e 849 [M+H].

15

The starting material was prepared as follows:

i) 0.5 g (2.37 mmol) of 2-(dichloromethyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane was dissolved in 10 ml of tetra-
20 hydrofuran and the solution was cooled under a nitrogen atmosphere to -78°C. 2.4 ml (2.4 mmol) of 1M propylmagnesium bromide were added dropwise and the solution was stirred at room temperature for 18 hours. The solution was partitioned between ethyl acetate, saturated sodium chloride solution and 2M
25 hydrochloric acid solution. The aqueous layer was extracted with ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulphate. After evaporation there was obtained 0.38 g of 2-[1(RS)-chlorobutyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane as an oil which was used in the next step without
30 further purification.

ii) 1.7 ml (1.7 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran were added dropwise to a solution of 0.37 g (1.69 mmol) of 2-[1(RS)-chlorobutyl]-4,4,5,5-tetramethyl-1,3,2-
35 dioxaborolane in 20 ml of tetrahydrofuran under nitrogen at -78°C. The solution was then stirred overnight at room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by

filtration and the filtrate was cooled to 0°C. 0.39 ml (5.1 mmol) of trifluoroacetic acid was added and the solution was stirred at 0°C for 30 minutes. The solution was evaporated and the residue was co-evaporated with toluene to give 0.62 g of α -(RS)-propyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate as a brown oil which was used in the next step without further purification.

iii) 0.2 g (0.218 mmol) of N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 6 ml of dichloromethane. 0.12 ml (1.1 mmol) of N-methylmorpholine was added and the solution was cooled to -15°C under a nitrogen atmosphere. 40 mg (0.31 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -15°C. 0.14 g (0.44 mmol) of α -(RS)-propyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate was added and the mixture was stirred at room temperature for 66 hours. After evaporation the residue was partitioned between ethyl acetate and 2M hydrochloric acid. The organic layer was washed with 2M hydrochloric acid, water and saturated sodium chloride solution and then dried over anhydrous sodium sulphate. After evaporation there was obtained 0.17 g of N²-[N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide in the form of a solid; NMR (DMSO, 400 MHz) δ : 0.75-0.9 (m,17H), 1.01-1.08 (m,6H), 1.15-1.25 (m,1H), 1.35 9s,36H), 1.4-1.7 (m,4H), 1.75-1.8 (m,1H), 2.05-2.15 (m,2H), 2.23 (s,3H), 2.29-2.41 (m,6H), 2.55-2.6 (m,1H), 2.7-2.74 (m,1H), 2.95-3.05 (m,1H), 4.15-4.25 (m,3H), 4.48-4.55 (m,1H), 4.6-4.7 (m,1H), 7.05-7.11 (m,4H), 7.7-7.81 (m,2H), 8.05-8.12 (m,2H), 8.15-8.25 (m,2H).

35 Example 8

0.126 g (0.116 mmol) of N²-[N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-

α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-
N1[3,3-difluoro-[1(S)-(dimethoxymethyl)-butyl]-L-leucinamide
was dissolved in 5 ml of trifluoroacetic acid and 5 ml of
dichloromethane. A few drops of water were added and the
5 solution was stirred at room temperature for 1 hour. The residue
was evaporated, the residue was triturated with diethyl ether and
the resulting solid was filtered off. The solid was purified by
chromatography on silica gel using dichloromethane/methanol/
acetic acid/water (75:15:3:2) for the elution. There were
10 obtained 67 mg of 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L-
 α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-
L-valyl]-L-leucyl]amino]-4,4-difluorovaleraldehyde as a cream
coloured solid of melting point 128-130°C.

15 The starting material was prepared as follows:

- i) 1.5 g (4.62 mmol) of 4,4-difluoro-L-norvaline p-toluene-
sulphonate were dissolved in dimethylformamide. 1.71 g
(7.85 mmol) of di-tert-butyl dicarbonate and 3.23 ml
20 (23.25 mmol) of triethylamine were added and the solution was
stirred at 60°C for 3 hours. The solution was evaporated and the
residue was partitioned between ethyl acetate and 2M hydro-
chloric acid. The organic layer was dried over anhydrous sodium
sulphate and evaporated. The resulting oil was purified by
25 chromatography on silica gel using ethyl acetate for the elution.
There were obtained 1.16 g of N-(tert-butoxycarbonyl)-4,4-
difluoro-L-norvaline as an orange oil which was used directly in
the next step.
- 30 ii) 1.16 g (4.62 mmol) of N-(tert-butoxycarbonyl)-4,4-
difluoro-L-norvaline were dissolved in 30 ml of dichloromethane.
6.4 ml (46.2 mmol) of triethylamine, 564 mg (4.62 mmol) of
N,N-dimethylaminopyridine, 1.77 g (9.24 mmol) of 1-(3-
dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and
35 1.8 g (18.5 mmol) of N,O-dimethylhydroxylamine hydrochloride
were added and the solution was stirred at room temperature for
18 hours. The mixture was diluted with ethyl acetate, washed
with 2M hydrochloric acid and aqueous sodium hydrogen carbonate

solution, dried over anhydrous sodium sulphate and evaporated to give an oil which was purified by chromatography on silica gel using ethyl acetate for the elution. There were obtained 547 mg of N,O-dimethyl 2(S)-(tert-butoxyformamido)-4,4-difluoro-
5 valerohydroxamate as a colourless oil; MS: m/e 297 [M+H].

- iii) 547 mg (1.85 mmol) of N,O-dimethyl 2(S)-(tert-butoxyformamido)-4,4-difluorovalerohydroxamate were dissolved in 12 ml of tetrahydrofuran and the solution was stirred at 0°C.
- 10 1.76 ml (1.76 mmol) of 1M lithium aluminium hydride in tetrahydrofuran were added and the solution was stirred for 15 minutes. The mixture was partitioned between ethyl acetate and saturated aqueous potassium hydrogen sulphate solution. The organic layer was evaporated and the residue was dissolved in
15 freshly prepared methanolic hydrogen chloride solution. After 1 hour the solution was evaporated to give 372 mg of 3,3-difluoro-1(S)-(dimethoxymethyl)butylamine hydrochloride as a white solid; MS: m/e 184 [M+H].
- 20 iv) 0.3 g (0.33 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 15 ml of dichloromethane. 0.22 ml (1.98 mmol) of N-methylmorpholine, 96 mg (0.5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 45 mg (0.33 mmol) of hydroxybenzotriazole and 217 mg (0.99 mmol) of 3,3-difluoro-1(S)-(dimethoxymethyl)butylamine hydrochloride
25 were added and the solution was stirred at room temperature for 18 hours. The mixture was washed with 2M hydrochloric acid and
30 aqueous sodium hydrogen carbonate solution, dried over anhydrous sodium sulphate and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried. There were obtained 143 g of N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-
35 [3,3-difluoro-1(S)-dimethoxymethyl)butyl]-L-leucinamide; MS: m/e 1106 [M+Na]⁺.

Example 9

80 mg (0.075 mmol) of N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-
[1(R)-dimethoxymethyl]-2-(methylthio)ethyl]-L-leucinamide
were dissolved in 10 ml of trifluoroacetic acid/dichloromethane
(1:1) containing 3 drops of water and the solution was stirred for
90 minutes under a nitrogen atmosphere. The solution was
10 evaporated to dryness under a vacuum and the residue was re-
evaporated twice with toluene. The solid was triturated with
10 ml of diethyl ether to give 60 mg of 2(R)-[N-[N-[N-[N-[N-(3-
carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-
phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(methylthio)-
15 propionaldehyde as a white solid; MS: m/e 851.5 [M+H]⁺.

The starting material was prepared as follows:

i) 2 g (8.51 mmol) of N-(tert-butoxycarbonyl)-S-methyl-L-
20 cysteine were dissolved in 60 ml of anhydrous tetrahydrofuran
and then 1.81 g (11.9 mmol) of 1-hydroxybenzotriazole hydrate,
2.28 g (11.88 mmol) of 1-(3-dimethylaminopropyl)-3-ethyl-
carbodiimide hydrochloride, 1.16 g (11.90 mmol) of N,O-
dimethylhydroxylamine hydrochloride and 5.9 ml (33.87 mmol) of
25 N,N-diisopropylethylamine were added. The mixture was stirred
overnight at room temperature. The solvent was removed by
evaporation and the residue was partitioned between ethyl
acetate and 5% (w/v) aqueous citric acid. The organic phase was
washed with saturated aqueous sodium bicarbonate solution and
30 then with saturated sodium chloride solution, dried over
magnesium sulphate and evaporated under a vacuum to give
2.27 g of N,O-dimethyl 2(R)-(tert-butoxyformamido)-3-(methyl-
thio)propionohydroxamate as a colourless oil; MS: m/e 279
[M+H]⁺.

35

ii) 2.22 g (7.90 mmol) of N,O-dimethyl 2(R)-(tert-butoxy-
formamido)-3-(methylthio)propionohydroxamate were dissolved
in 25 ml of anhydrous tetrahydrofuran and the solution was

cooled to 0°C. 4.69 ml (4.69 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran were added dropwise and the mixture was stirred for 15 minutes. The reaction was quenched by the dropwise addition of saturated aqueous potassium hydrogen sulphate solution and then 50 ml of diethyl ether were added. The mixture was stirred vigorously for 20 minutes. The organic phase was separated, washed with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated to give 1.75 g of aldehyde which, without further purification, was dissolved in 20 ml of saturated methanolic hydrogen chloride solution and stirred for 2 hours under a nitrogen atmosphere at room temperature. The solvent was removed by evaporation and the residue was re-evaporated twice with toluene to give 1.3 g of dimethyl acetal as a colourless oil.

90 mg (0.45 mmol) of the dimethyl acetal were dissolved in 40 ml dichloromethane and then 200 mg (0.22 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 100 mg (0.87 mmol) of N-ethylmorpholine, 40 mg (0.26 mmol) of 1-hydroxybenzotriazole hydrate and 50 mg (0.26 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. The solution was stirred overnight at room temperature. The organic phase was washed with 5% (w/v) aqueous citric acid and then with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated under a vacuum. The resulting oil was triturated with 10 ml of diethyl ether to give 165 mg of N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-(dimethoxymethyl)-2-(methylthio)ethyl]-L-leucinamide as a white solid; MS: m/e 1065.7 [M+H]⁺.

Example 10

50 mg (0.048 mmol) of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-3-butenyl]-L-leucinamide were dissolved in 4 ml of trifluoroacetic acid/dichloromethane (1:1) containing 3 drops of water and the solution was stirred for 1 hour under nitrogen. The solution was evaporated to dryness
10 under a vacuum and the residue was re-evaporated twice with toluene. The solid was triturated with 10 ml of diethyl ether to give 30 mg of 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentenaldehyde; MS: m/e 831.5 [M+H]⁺.
15

The starting material was prepared as follows:

i) 1.13 g (7.46 mmol) of L-allylglycine hydrochloride were dissolved in 20 ml of saturated aqueous sodium bicarbonate
20 solution and 20 ml of dioxan. 1.95 g (8.93 mmol) of di-tert-butyl dicarbonate were added and the solution was stirred overnight and then evaporated to dryness under a vacuum. The residue was partitioned between diethyl ether and water. The aqueous phase was acidified with 2M hydrochloric acid and
25 extracted with ethyl acetate. The organic phase was dried over magnesium sulphate and evaporated under a vacuum to give 1.6 g of N-(tert-butoxycarbonyl)-L-allylglycine as a colourless oil. ¹H NMR (250 MHz, CDCl₃) δ : 1.4 (s,9H), 2.4-2.7 (m,2H), 4.3-4.5 (m,1H), 5.0 (br.d,1H), 5.1-5.2 (m,2H), 5.6-5.8 (m,1H)
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ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-4-penteno-hydroxamate was obtained in a manner analogous to that described in Example 10 i) from 1.6 g (7.44 mmol) of N-(tert-butoxycarbonyl)-L-allylglycine, 1.4 g (10.4 mmol) of 1-hydroxy-
35 benzotriazole, 1.99 g (10.4 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride, 1.02 g (10.46 mmol) of N,O-dimethylhydroxylamine hydrochloride and 2.6 ml (14.93 mmol) of ethyl diisopropylamine. This gave 1.9 g of

product as a colourless oil. ^1H NMR (250 MHz, CDCl_3) δ : 1.4 (s,9H), 2.3-2.6 (m,2H), 3.2 (s,3H), 3.8 (s,3H), 4.6-4.7 (m,1H), 5.0-5.4 (m,3H), 5.6-5.8 (m,1H).

5 iii) 1.9 g (7.36 mmol) of N,O-dimethyl 2(S)-(tert-butoxy-formamido)-4-pentenohydroxamate were dissolved in 20 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C . 5.40 ml (5.4 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran were added dropwise and the mixture
10 was stirred for 25 minutes. The reaction was quenched by the dropwise addition of saturated aqueous potassium hydrogen sulphate and then 50 ml of diethyl ether were added. The mixture was stirred vigorously for 20 minutes. The organic phase was separated, washed with saturated aqueous sodium
15 bicarbonate solution, dried over magnesium sulphate and evaporated to give the aldehyde which, without further purification, was dissolved in 25 ml of saturated methanolic hydrogen chloride solution and stirred for 2 hours at room temperature. The solvent was removed by evaporation and the
20 residue was re-evaporated twice with toluene to give the amino acid acetal as a brown oil.

iv) 40 mg (0.22 mmol) of the amino acid acetal were dissolved in 4 ml of dichloromethane and then 200 mg (0.22 mmol) of N-
25 [N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 0.1 ml (0.78 mmol) of N-ethylmorpholine, 35 mg (0.22 mmol) of 1-hydroxybenzotriazole monohydrate and 50 mg (0.26 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride were added. The
30 solution was stirred overnight at room temperature. The organic phase was washed with 5% (w/v) aqueous citric acid solution and then with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated under a vacuum. The
35 resulting oil was triturated with 10 ml of diethyl ether to give 148 mg of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-

3-butenyl]-L-leucinamide as a white solid; MS: m/e 1013.6 [M+H-MeOH]⁺.

Example 11

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90 mg (0.081 mmol) of N²-[N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[2-(butylthio)-1(R)-(dimethoxymethyl)ethyl]-L-leucinamide were
10 dissolved in 10 ml of trifluoroacetic acid/dichloromethane (1:1) containing 3 drops of water and the solution was stirred for 90 minutes under nitrogen. The solution was evaporated to dryness under a vacuum and the residue was re-evaporated twice with toluene. The solid was triturated with 10 ml of diethyl
15 ether to give 80 mg of 2(R)-[[N-[N-[N-[N-[N-(3-carboxy-propionyl)-L- α -aspartyl-L- α -glutamyl]-2-methyl-L-phenyl-alanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(butylthio)propion-aldehyde as a white solid. MS: m/e 893.4 [M+H]⁺.

20 The starting material was prepared as follows:

i) 2 g (16.53 mmol) of L-cysteine were dissolved in 40 ml of water/ethanol (1:1) together with 1.33 g (33.25 mmol) of sodium hydroxide pellets. 3.04 g (16.53 mmol) of butyl iodide
25 were added and the mixture was stirred for 2 hours. The resulting S-alkylated product was treated with 3.96 g (18.14 mmol) of di-tert-butyl dicarbonate and the mixture was stirred for 1 hour. A further 3.61 g (16.53 mmol) of di-tert-butyl dicarbonate were added and the mixture was stirred
30 overnight. The solution was evaporated to dryness under a vacuum and the residue was partitioned between diethyl ether and saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was acidified by partitioning in 2M hydrochloric acid and ethyl acetate, the separated organic phase was dried
35 over magnesium sulphate and the solvent was removed by evaporation to give 4.3 g of N-(tert-butoxycarbonyl)-S-butyl-L-cysteine as a brown oil; ¹H NMR (250 MHz, CDCl₃) δ : 0.9 (t,3H), 1.3-1.6 (m,4H), 1.4 (s,9H), 2.55 (t,2H), 3.0 (br.d,2H), 4.5 (m,1H),

5.3 (br.d,1H).

ii) N,O-Dimethyl 2(R)-(tert-butoxyformamido)-3-(butylthio)-propionohydroxamate was obtained in a manner analogous to that described in Example 10 i) from 2.15 g (7.76 mmol) of N-(tert-butoxycarbonyl)-S-butyl-L-cysteine, 1.19 g (7.77 mmol) of 1-hydroxybenzotriazole monohydrate, 2.24 g (11.68 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 1.14 g (11.68 mmol) of N,O-dimethylhydroxylamine hydrochloride and 1.34 g (11.64 mmol) of N-ethylmorpholine in 30 ml of dichloromethane. This gave 2.0 g of product as a colourless oil after column chromatography using ethyl acetate/petrol (1:2) as the eluent. ¹H NMR (250 MHz, CDCl₃) δ: 0.9 (t,3H), 1.3-1.6 (m,4H), 1.4 (s,9H), 2.55 (t,2H), 2.6 -2.7 (dd,1H), 2.8-2.9 (dd,1H), 3.2 (s,3H), 3.75 (s,3H), 4.8-4.9 (m,1H), 5.3 (br.d,1H).

iii) 0.3 g (0.94 mmol) of N,O-dimethyl 2(R)-(tert-butoxyformamido)-3-(butylthio)propionohydroxamate was dissolved in 10 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C. 0.55 ml (0.55 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran was added dropwise and the mixture was stirred for 15 minutes. The reaction was quenched by the dropwise addition of saturated aqueous potassium hydrogen sulphate and then 20 ml of diethyl ether were added. The mixture was stirred vigorously for 20 minutes. The organic phase was separated, washed with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated to give the aldehyde which, without further purification, was dissolved in 20 ml of saturated methanolic hydrogen chloride solution and stirred for 2 hours under a nitrogen atmosphere at room temperature. The solvent was removed by evaporation and the residue was re-evaporated twice with toluene to give the amino acid acetal as a brown oil.

200 mg (0.82 mmol) of the amino acid acetal were dissolved in 40 ml of dichloromethane and then 200 mg (0.22 mmol) of [N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L-α-aspartyl]-O-tert-butyl-L-α-

glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 100 mg (0.87 mmol) of N-ethylmorpholine, 40 mg (0.26 mmol) of 1-hydroxybenzotriazole and 50 mg (0.26 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. The solution was stirred for 2 hours at room temperature. The organic phase was washed with 5% (w/v) aqueous citric acid solution and then with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated under a vacuum. The resulting oil was triturated with 10 ml of diethyl ether to give 160 mg of N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[2-(butylthio)-[1(R)-(dimethoxymethyl)ethyl]-L-leucinamide as a white solid; MS: m/e 1075.6 [M+H-MeOH]⁺.

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Example 12

56 mg (0.049 mmol) of N1-[2-(benzylthio)-1(R)-(dimethoxymethyl)ethyl]-N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucinamide were dissolved in 10 ml of trifluoroacetic acid/dichloromethane (1:1) containing 3 drops of water and the solution was stirred for 90 minutes. The solution was evaporated to dryness under a vacuum and the residue was re-evaporated twice with toluene. The solid was triturated with 10 ml of diethyl ether to give 40 mg of 3-(benzylthio)-2(R)-[[N-[N-[N-[N-[3-carboxypropionyl]-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propionaldehyde as a white solid. MS: m/e 927.6 (M+H)⁺.

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The starting material was prepared as follows:

- i) S-Benzyl-N-(tert-butoxycarbonyl)-L-cysteine was obtained in a manner analogous to that described in Example 10 i) from 1 g (4.74 mmol) of S-benzyl-L-cysteine, 0.8 g (9.5 mmol) of sodium bicarbonate and 1.4 g (6.4 mmol) of di-tert-butyl dicarbonate. There were obtained 1.4 g of a colourless oil; ¹H NMR (250 MHz,

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CDCl_3) δ : 1.4 (s,9H), 2.8-2.9 (m,2H), 3.7 (s,2H), 4.4-4.5 (m,1H), 5.3 (d,1H), 7.2-7.4 (m,5H)

ii) N,O-Dimethyl 3-(benzyl)-2(R)-(tert-butoxyformamido)-
5 propionohydroxamate was obtained in a manner analogous to that described in Example 9 i) from 1.4 g (4.52 mmol) of S-benzyl-N-(tert-butoxycarbonyl)-L-cysteine, 0.70 g (4.6 mmol) of 1-hydroxybenzotriazole monohydrate, 1.30 g (6.77 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.66 g
10 (6.77 mmol) of N,O-dimethylhydroxylamine hydrochloride and 0.78 g (6.77 mmol) of N-ethylmorpholine in 40 ml of dichloromethane. There were obtained 0.60 g of a colourless oil; ^1H NMR (250 MHz, CDCl_3) δ : 1.4 (s,9H), 2.55-2.65 (dd,1H), 2.75-2.85, (dd,1H), 3.2 (s,3H), 3.7 (s,3H), 3.72 (s,2H), 4.9 (m,1H), 5.3 (d,1H),
15 7.2 -7.35 (m,5H).

iii) 0.48 g (1.36 mmol) of N,O-dimethyl 3-(benzyl)-2(R)-(tert-butoxyformamido)propionohydroxamate was dissolved in 10 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C .
20 0.95 ml (0.95 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran was added dropwise and the mixture was stirred for 15 minutes. The reaction was quenched by the dropwise addition of saturated aqueous potassium hydrogen sulphate and then 20 ml of diethyl ether were added. The
25 mixture was stirred vigorously for 20 minutes. The organic phase was separated, washed with saturated aqueous sodium bicarbonate solution, dried magnesium sulphate and evaporated to give the aldehyde which, without further purification, was dissolved in 10 ml of saturated methanolic hydrogen chloride
30 solution and stirred for 2 hours at room temperature. The solvent was removed by evaporation and the residue was re-evaporated twice with toluene to give the amino acid acetal as a brown oil.

35 100 mg (0.36 mmol) of the amino acid acetal were dissolved in 40 ml of dichloromethane and then 200 mg (0.22 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)-propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -

glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 100 mg (0.87 mmol) of N-ethylmorpholine, 40 mg (0.30 mmol) of 1-hydroxybenzotriazole and 50 mg (0.26 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. The solution was stirred overnight at room temperature. The organic phase was washed with 5% (w/v) aqueous citric acid solution and then with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated under a vacuum. The resulting oil was triturated with 10 ml of diethyl ether to give 160 mg of N1-[2-(benzylthio)-1(R)-(dimethoxymethyl)ethyl]-N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucinamide as a white solid; MS: m/e 1109.8 [M+H-MeOH]⁺.

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Example 13

49 mg (0.046 mmol) of N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-3-pentynyl]-L-leucinamide were dissolved in 4 ml of trifluoroacetic acid/dichloromethane (1:1) containing 3 drops of water and the solution was stirred for 1 hour under a nitrogen atmosphere. The solution was evaporated to dryness under a vacuum and the residue was re-evaporated twice with toluene. The solid was triturated with 10 ml of diethyl ether to give 30 mg of 2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexynal as a white solid; MS: m/e 843.6 [M+H]⁺.

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The starting material was prepared as follows:

i) N-(tert-Butoxycarbonyl)-L-(2-butyrynyl)glycine was obtained in a manner analogous to that described in Example 10 i) from 1.0 g (7.80 mmol) of L-(2-butyrynyl)glycine (prepared according to Sasaki et al. Int. J. Peptide Protein Res 1986, 27, 360-365), 2.66 g (31.7 mmol) of sodium bicarbonate and 1.89 g

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(8.66 mmol) of di-tert-butyl dicarbonate. There was obtained 1.94 g of a colourless oil; ^1H NMR (250 MHz, CDCl_3) δ : 1.45 (s,9H), 1.75 (t,3H), 2.6-2.9 (m,2H), 4.4-4.5 (m,1H), 5.3 (br.d,1H).

- 5 ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-4-hexyno-
hydroxamate was obtained in a manner analogous to that
described in Example 9 i) from 1.74 g (7.67 mmol) of N-(tert-
butoxycarbonyl)-L-(2-butynyl)glycine, 1.45 g (9.5 mmol) of 1-
hydroxybenzotriazole, 2.06 g (10.73 mmol) of 1-(3-dimethyl-
10 aminopropyl)-3-ethylcarbodiimide hydrochloride, 1.05 g
(10.77 mmol) of N,O-dimethylhydroxylamine hydrochloride and
5.3 ml (30.43 mmol) of ethyldiisopropylamine in 80 ml of
tetrahydrofuran. There were obtained 2.0 g of a colourless oil;
 ^1H NMR (250 MHz, CDCl_3) δ : 1.4 (s,9H), 1.75 (t,3H), 2.55 (m,2H), 3.2
15 (s,3H), 3.5 (s,3H), 4.7-4.8 (m,1H), 5.35 (br.d,1H).

- iii) 1.0 g (3.70 mmol) of N,O-dimethyl 2(S)-(tert-butoxy-
formamido)-4-hexynohydroxamate was dissolved in 10 ml of
anhydrous tetrahydrofuran and the solution was cooled to 0°C.
20 2.59 ml (2.59 mmol) of a 1M solution of lithium aluminium
hydride in tetrahydrofuran were added dropwise and the mixture
was stirred for 30 minutes. The reaction was quenched by the
dropwise addition of 20 ml of saturated aqueous potassium
hydrogen sulphate and then 50 ml of diethyl ether were added.
25 The mixture was stirred vigorously for 30 minutes. The organic
phase was separated, washed with saturated aqueous sodium
bicarbonate solution, dried over magnesium sulphate and evapor-
ated to give the aldehyde which, without further purification,
was dissolved in 10 ml of saturated methanolic hydrogen
30 chloride solution and stirred for 2 hours under a nitrogen atmos-
phere at room temperature. The solvent was removed by evapor-
ation and the residue was re-evaporated twice with toluene to
give the amino acid acetal as a brown oil.

- 35 47 mg (0.24 mmol) of the amino acid acetal were dissolved
in 20 ml of dichloromethane and then 200 mg (0.22 mmol) of N-
[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L-
 α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenyl-

alanyl]-3-methyl-L-valyl]-L-leucine, 0.1 ml (0.78 mmol) of N-ethylmorpholine, 42 mg (0.27 mmol) of 1-hydroxybenzotriazole and 59 mg (0.31 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride were added. The solution was stirred overnight at room temperature. The organic phase was washed with 5% (w/v) aqueous citric acid solution and then with saturated aqueous sodium bicarbonate solution, dried over magnesium sulphate and evaporated under a vacuum. The resulting oil was triturated with 10 ml of diethyl ether to give 110 mg of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-3-pentynyl]-L-leucinamide as a white solid. MS: m/e 1025.8 [M+H-MeOH]⁺.

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Example 14

0.065 g (0.06 mmol) of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(RS)-(dimethoxymethyl)-2-(3-thienyl)ethyl]-L-leucinamide was dissolved in 10 ml of dichloromethane/trifluoroacetic acid (1:1) containing 3 drops of water. The solution was stirred for 3 hours at room temperature. After removal of the solvent by evaporation the crude product was chromatographed on silica gel using dichloromethane:methanol:acetic acid:water (120:15:3:2) as the eluent to give 0.035 g of 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3(3-thienyl)propionaldehyde as a white solid; MS: m/e 887.7 [M+H]⁺.

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The starting material was prepared as follows:

i) 0.5 g (2.92 mmol) of 3-(3-thienyl)-DL-alanine was dissolved in 15 ml of water and 15 ml of dioxan. 2.5 g (29.76 mmol) of sodium hydrogen carbonate and 3.53 g (16.19 mmol) of di-tert-butyl dicarbonate were added and the solution was stirred for 2 hours and then evaporated to dryness

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- under a vacuum. The residue was partitioned between diethyl ether and saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was acidified with 2M hydrochloric acid and extracted with ethyl acetate. The organic phase was dried over magnesium sulphate and the solvent was evaporated under a vacuum to give 0.685 g of N-(tert-butoxycarbonyl)-3-(3-thienyl)-DL-alanine as a colourless oil; ^1H NMR (250 MHz, CDCl_3) δ : 1.4 (s,9H), 2.9 (dd,1H), 3.15 (dd,1H), 4.3 (m,1H), 7.0 (d,1H), 7.1 (br s,H), 7.3 (m,1H).
- ii) 0.69 g (2.55 mmol) of N-(tert-butoxycarbonyl)-3-(3-thienyl)-DL-alanine was dissolved in 40 ml of dichloromethane. 0.34 g (3.56 mmol) of N,O-dimethylhydroxylamine hydrochloride, 0.54 g (3.53 mmol) of 1-hydroxybenzotriazole monohydrate, 0.68 g (3.55 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 1.0 g (8.70 mmol) of 4-ethylmorpholine were added and the resulting solution was stirred at room temperature overnight. The solution was then washed with 5% citric acid solution, saturated sodium hydrogen carbonate solution and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After evaporation of the solvent the crude product was chromatographed on silica gel using 30% ethyl acetate in petroleum ether as the eluent to give 0.75 g of N,O-dimethyl 2(RS)-(tert-butoxyformamido)-3-(3-thienyl)-propionohydroxamate as a white solid; ^1H NMR (250 MHz, CDCl_3) δ : 1.4 (s,9H), 2.95 (dd,1H), 3.05 (dd,1H), 3.15 (s,3H), 3.65 (s,3H), 4.9 (m,1H), 5.15 (br d,1H), 6.9 (d,1H), 7.0 (d,1H), 7.2 (m,1H).
- iii) 0.2 g (0.64 mmol) of N,O-dimethyl 2(RS)-(tert-butoxyformamido)-3-(3-thienyl)propionohydroxamate was dissolved in 10 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C. 0.5 ml (0.5 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran was added dropwise and the solution was stirred for 15 minutes. The reaction was quenched by the dropwise addition of saturated potassium hydrogen sulphate solution and then 30 ml of diethyl ether were added. The resulting two phase system was stirred vigorously for 1 hour. The organic phase was separated, washed with saturated sodium

hydrogen carbonate solution and saturated sodium chloride solution, dried over magnesium sulphate and evaporated to give the aldehyde which, without purification, was dissolved in 10 ml of a saturated methanolic hydrogen chloride solution and stirred
5 at room temperature for 2 hours. After removal of the solvent by evaporation the dimethyl acetal was used in the next step without purification.

The dimethyl acetal was dissolved in 40 ml of dichloro-
10 methane and then 0.15 g (0.16 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 0.03 g (0.2 mmol) of 1-hydroxybenzotriazole, 0.038 g (0.2 mmol) of 1-(3-dimethylaminopropyl)-3-ethyl-
15 carbodiimide hydrochloride and 0.08 g (0.65 mmol) of N-ethylmorpholine were added and the resulting solution was stirred at room temperature for 2 hours. The solution was washed with 5% citric acid solution, saturated sodium hydrogen carbonate solution and saturated sodium chloride solution and dried over
20 anhydrous magnesium sulphate. After removal of the solvent by evaporation the crude product was chromatographed on silica gel using 5% methanol in dichloromethane as the eluent to give 0.07 g of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(RS)-(dimethoxy-
25 methyl)-2-(3-thienyl)ethyl]-L-leucinamide as a white solid; MS: m/e 1069 [M+H-MeOH]⁺.

Example 15

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0.08 g (0.07 mmol) of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-2-(2-thienyl)ethyl]-L-leucinamide was
35 dissolved in 10 ml of dichloromethane/trifluoroacetic acid (1:1) containing 3 drops of water and the solution was stirred for 2 hours at room temperature. After removal of the solvent by evaporation the crude product was chromatographed on silica gel

using dichloromethane:methanol:acetic acid:water (120:15:3:2) as the eluent to give 0.021 g of 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3(2-thienyl)propionaldehyde as a white solid; MS: m/e 887.4 [M+H]⁺.

The starting material was prepared as follows:

- i) 0.63 g (2.33 mmol) of N-(tert-butoxycarbonyl)-3-(2-thienyl)-L-alanine was dissolved in 50 ml of dichloromethane and then 0.34 g (3.48 mmol) of N,O-dimethylhydroxylamine hydrochloride, 0.36 g (2.35 mmol) of 1-hydroxybenzotriazole monohydrate, 0.67 g (3.49 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.40 g (3.47 mmol) of N-ethylmorpholine were added. The resulting solution was stirred at room temperature overnight. The solution was washed with 5% citric acid, then with saturated sodium hydrogen carbonate solution and saturated sodium chloride solution, dried over anhydrous magnesium sulphate and evaporated to give 0.70 g of N,O-dimethyl 2(S)-(tert-butoxyformamido)-3-(2-thienyl)propionohydroxamate as a white solid; ¹H NMR (250 MHz, CDCl₃) δ : 1.4 (s,9H), 3.1 (dd,1H), 3.15 (s,3H), 3.2 (dd,1H), 3.7 (s,3H), 4.9 (br d,1H), 5.8 (m,1H), 6.8 (d,1H), 6.9 (dd,1H), 7.15 (d,1H).
- ii) 0.4 g (1.27 mmol) of N,O-dimethyl 2(S)-(tert-butoxyformamido)-3-(2-thienyl)propionohydroxamate was dissolved in 10 ml of anhydrous tetrahydrofuran and the solution was cooled to 0°C. 0.9 ml (0.9 mmol) of a 1M solution of lithium aluminium hydride in tetrahydrofuran was added and the resulting solution stirred for 15 minutes. The reaction was quenched by the dropwise addition of 15 ml of saturated potassium hydrogen sulphate solution and then 30 ml of diethyl ether were added. The resulting two phase system was stirred vigorously for 40 minutes. The organic phase was separated, washed with saturated sodium hydrogen carbonate solution and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation the aldehyde, without further purification, was dissolved in 10 ml of

a saturated methanolic hydrogen chloride solution and stirred at room temperature for 2 hours. After removal of the solvent by evaporation the dimethyl acetal was used in the next step without purification.

5

The dimethyl acetal was dissolved in 40 ml of dichloromethane and then 0.20 g (0.22 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 0.04 mg (0.26 mmol) of 1-hydroxybenzotriazole, 0.05 g (0.26 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.10 g (0.87 mmol) of N-ethylmorpholine were added. The resulting solution was stirred at room temperature for 2 hours, then washed in sequence with 5% citric acid solution, saturated sodium hydrogen carbonate solution and saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation the crude product was triturated with diethyl ether to give 0.16 g of N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-2-(2-thienyl)ethyl-L-leucinamide as a white solid. MS: m/e 1069.6 [M+H-MeOH]⁺.

25

Example 16

In an analogous manner to that described in Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-L-cyclohexylglycine there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L-2-cyclohexylglycyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutylaldehyde as a white solid; MS: m/e 883.5 [M+H].

35

Example 17

In an analogous manner to that described in Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-

alanine with N-[(9-fluorenyl)methoxycarbonyl]-L-valine there was obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid;
5 MS: m/e 843.5 [M+H].

Example 18

In an analogous manner to that described in Example 4, by
10 replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-D-alanine there was obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-alanyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white
15 solid; MS: m/e 815.4 [M+H].

Example 19

In an analogous manner to that described in Example 4, by
20 replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-D-valine there was obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid;
25 MS: m/e 843.4 [M+H].

Example 20

0.2 g (0.2 mmol) of N²-[N-[N-[N-(carboxypropionyl)-L-
30 α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)pentyl]-L-leucinamide was dissolved in 12 ml of acetone and 12 ml of 0.1M ammonium acetate in water were added. 0.21 g (1 mmol) of
35 sodium periodate was added and the resulting mixture was stirred at room temperature for 22 hours. 7 ml of water were then added together with a small amount of sodium periodate. The resulting solution was stirred for a further 5 hours. The

acetone was removed under a vacuum and the aqueous residue was acidified with 2N hydrochloric acid and then extracted with ethyl acetate. Saturated aqueous sodium chloride was added to the aqueous layer which was then extracted with ethyl acetate. The organic extracts were combined, dried over sodium sulphate and evaporated. The residue was triturated with diethyl ether, filtered off and dried to give 167 mg of 1(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]pentylboronic acid as a white solid; MS: m/e 845.4 [M+H-H₂O]⁺.

The starting material was prepared as follows:

- i) In an analogous manner to that described in Example 21 i) and ii), by replacing 3-butenylmagnesium bromide with butylmagnesium bromide there was obtained α -(R)-butyl-3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole-2-methylamine trifluoroacetate (1:1) which was used in the next step without further purification.
- ii) 0.25 g (0.27 mmol) of N-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 4 ml of dichloromethane. 0.15 ml (1.4 mmol) of N-methylmorpholine was added and the solution was cooled to -10°C under a nitrogen atmosphere. 45 mg (0.32 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -10°C. 0.2 g (0.54 mmol) of α -(R)-butyl-3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole-2-methylamine trifluoroacetate (1.1) was added and the mixture was stirred at room temperature for 16 hours. The solution was diluted with dichloromethane, washed with 2M hydrochloric acid and water and dried over anhydrous sodium sulphate. After evaporation the residue was triturated with diethyl ether and dried. There was obtained 0.227 g of N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-

L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S), 4(S),5,6(S), 7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)pentyl]-L-leucinamide as a white solid; MS: m/e 1165.9 [M+H]⁺.

5

iii) 300 mg (0.26 mmol) of N2-[N-[N-[N-(tert-butoxy-carbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide were dissolved in 3.5 ml of trifluoroacetic acid and 3.5 ml of dichloromethane. The solution was stirred at room temperature for 45 minutes, then diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried and then purified by chromatography on silica gel using dichloromethane/methanol/acetic acid/water (170:15:3:2) for the elution. There were obtained 135 mg of N2-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S), 4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide as a white solid: MS: m/e 995.3 [M+H]⁺.

Example 21

25

N2-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide can be converted into N2-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentenylboronic acid in an analogous manner to that described in the first paragraph of Example 20.

35

The starting material was prepared as follows:

i) 0.5 g (1.9 mmol) of 2-(dichloromethyl)-3a(S),4(S),5,6(S), 7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzo-

- dioxaborole was dissolved in 5 ml of tetrahydrofuran and the solution was cooled to -78°C under a nitrogen atmosphere. 4.5 ml (2.3 mmol) of 0.5M 3-butenylmagnesium bromide in tetrahydrofuran were added dropwise and the resulting solution
- 5 was stirred for 2 minutes. 3 ml (1.52 mmol) of 0.5M zinc (II) chloride solution were then added and the mixture was stirred for 16 hours while slowly warming to room temperature. The mixture was diluted with ethyl acetate and then washed with 2M hydrochloric acid and brine. The organic phase was dried over
- 10 sodium sulphate and then evaporated under a vacuum. The residue was purified by chromatography on silica gel using diethyl ether/hexane (1:9) for the elution to give 177 mg of 2-[1(S)-chloro-4-pentenyl]-3a(S)-4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole. NMR: (CDCl₃)
- 15 0.83 (s, 3H), 1.15 (d, 1H), 1.30 (s, 3H), 1.42 (s, 3H), 1.42 (s, 3H), 1.85-1.95 (m, 4H), 2.08 (t, 1H), 2.15-2.35 (m, 4H), 3.49 (dd, 1H), 4.35 (dd, 1H), 5.0 (dd, 1H), 5.07 (dd, 1H), 5.78 (m, 1H).
- ii) 0.158 g (0.56 mmol) of 2-[1(S)-chloro-4-pentenyl]-
- 20 (3a(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole was dissolved in 2 ml of tetrahydrofuran and then cooled to -78°C under a nitrogen atmosphere. 0.56 ml (0.56 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran was added dropwise. The solution was then stirred
- 25 overnight while slowly warming to room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration. The solvent was removed by evaporation, the residue was dissolved in 2 ml of diethyl ether and the solution was cooled to
- 30 0°C. 0.12 ml (1.7 mmol) of trifluoroacetic acid was added and the solution was stirred at 0°C for 30 minutes. The solution was evaporated and the residue was co-evaporated with toluene to give 0.0226 g of a-(R)-(3-butenyl)-3a(S),4(S),5,6(S),7, 7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborole-2-methylamine trifluoroacetate (1:1) as an oil which was
- 35 used in the next step without further purification.

- iii) 0.35 g (0.38 mmol) of N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 6 ml of dichloromethane.
- 5 0.21 ml (1.9 mmol) of N-methylmorpholine was added and the solution was cooled to -15°C under a nitrogen atmosphere. 66 mg (0.46 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -15°C. 0.2 g (0.53 mmol) of α -(R)-(3-butenyl)-3a(S),4(S),5,6(S),7,7a(R)-
- 10 hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzoxaborole-2-methylamine trifluoroacetate (1.1) was added and the mixture was stirred at room temperature for 5 hours. The solution was diluted with dichloromethane, washed with 2M hydrochloric acid and water and dried over anhydrous sodium sulphate. After
- 15 evaporation the residue was triturated with diethyl ether and dried. There was obtained 0.309 g of N2-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-
- 20 [1(R)-(3a(S),4(S),5,6(S),7,7a(S)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide as a solid which was used without further purification.
- iv) 300 mg (0.26 mmol) of N2-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -
- 25 glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide were dissolved in 3.5 ml of trifluoroacetic acid and 3.5 ml of dichloromethane. The solution was stirred at room temperature
- 30 for 45 minutes, then diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried and then purified by chromatography on silica gel using dichloromethane/methanol/acetic acid/water (170:15:3:2) for the elution. There were obtained 135 mg of N2-
- 35 [N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-4-pentenyl]-L-leucinamide as a

white solid: MS: m/e 995.3 [M+H]⁺.

Example 22

5 N2-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -
glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-
[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-
methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-leucinamide can
be converted into 1(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -
10 aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-
valyl]-L-leucyl]amino]propylboronic acid in a manner analogous to
that described in the first paragraph of Example 20.

The starting material was prepared as follows:

15

i) In an analogous manner to that described in Example 21 i)
and ii), by replacing 3-butenylmagnesium bromide with ethyl-
magnesium bromide there was obtained α (R)-ethyl-3a(R)-ethyl-
3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-
20 methano-1,3,2-benzodioxaborole-2-methylamine trifluoroacetate
(1:1) which was used in the next step without further
purification.

ii) 0.35 g (0.38 mmol) of N-[N-[N-[N-(tert-butoxy-
25 carbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -
glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-valyl]-
L-leucine was dissolved in 3 ml of dimethylformamide and 7 ml
of dichloromethane. 0.2 ml (1.9 mmol) of N-methylmorpholine
was added and the solution was cooled to -10°C under a nitrogen
30 atmosphere. 68 mg (0.53 mmol) of isobutyl chloroformate were
added and the solution was stirred for 10 minutes at -10°C.
0.18 g (0.53 mmol) of α (R)-ethyl-3a(S)4(S),5,6,(S),7,7a(R)-
hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxo-
borole-2-methylamine trifluoroacetate (1:1) was added and the
35 mixture was stirred at room temperature for 16 hours. After
evaporation the residue was partitioned between ethyl acetate
and 2M hydrochloric acid. The organic layer was washed with
water and saturated sodium chloride solution and then dried over

- anhydrous sodium sulphate. The solution was evaporated and the residue was triturated with diethyl ether, filtered off and dried to give 0.22 g of N²-[N-[N-[N-[3-(tert-butoxycarbonyl)-propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-leucinamide as a solid which was used without further purification.
- iii) 0.22 g (0.19 mmol) of N²-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-leucinamide was dissolved in 5 ml of trifluoroacetic acid and 5 ml of dichloromethane, the solution was stirred at room temperature for 1 hour and then diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried to give 170 mg of N²-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-(3a(S),4(S),5,6(S),7,7a(R)-hexahydro-3a, 5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)propyl]-L-leucinamide as a white solid; MS: m/e 969.4 [M+H]⁺.

Example 23

- 4 g of 0.25 mmol/g 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methyl-N-[α (RS)-(4-methylphenyl)benzyl]valeramide-polystyrene conjugate were swollen in dimethylformamide for 20 minutes and then suspended and agitated in dimethylformamide/piperidine (4.1). After 5 minutes the resin was drained and then suspended in and agitated with dimethylformamide/piperidine (4.1) for a further 5 minutes. The resin was then drained and washed five times with dimethylformamide.

The resin was then suspended in a solution of 2.1 g (6 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valine in dimethylformamide and then a mixture of 1.9 g of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 1.3 ml of N-methylmorpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed five times with dimethylformamide.

10 The resin was resuspended in and agitated with dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethylformamide/piperidine (4:1) for a further 5 minutes. Then, the residue was drained and washed five times with dimethyl formamide.

15 The resin was then suspended in a solution of 2.4 g (6 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-3-(2-methylphenyl)-L-alanine in dimethylformamide and a mixture of 1.9 g of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 1.3 ml of N-methylmorpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed five times with dimethyl formamide.

25 40 mg of this resin were resuspended in and agitated with 0.7 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethylformamide/ piperidine (4:1) for a further 5 minutes. Then, the resin was drained and washed five times with dimethylformamide.

35 The resin was then suspended in 0.5 ml of a 0.2M solution of N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamic acid in dimethyl sulfoxide and then 0.5 ml of a mixture of 0.2M 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.4M N-methylmorpholine in dimethylformamide was added. After agitating for 1 hour the resin was drained and washed five times with 1 ml of dimethylformamide

The resin was resuspended in and agitated with 0.7 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethyl-
5 formamide/piperidine (4:1) for a further 5 minutes. Then, the resin was drained and washed five times with 1 ml of dimethylformamide.

The resin was suspended in 0.5 ml of a solution of N-(9-
10 fluorenylmethoxycarbonyl)-O-tert-butyl-L-tyrosine in dimethyl sulphoxide and 0.5 ml of a mixture of 0.2M 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.4M N-methylmorpholine in dimethylformamide was added. After
agitating for 1 hour the resin was drained and washed five times
15 with 1 ml of dimethylformamide.

The resin was resuspended in and agitated with 0.7 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and resuspended in and agitated with dimethyl-
20 formamide/piperidine (4:1) for a further 5 minutes. Then, the resin was drained and washed five times with 1 ml of dimethylformamide.

The residue was suspended in 0.5 ml of a 0.2M solution of
25 tert-butyl hydrogen succinate in dimethylformamide and then 0.5 ml of 0.2M 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.4M N-methylmorpholine dissolved in dimethylformamide was added. After agitating for
1 hour the resin was drained and washed five times with 1 ml of
30 dimethylformamide and then twice with 1 ml of dichloromethane.

0.2 ml of dichloromethane was added to the resin which was then treated with 0.7 ml of trifluoroacetic acid/water (19:1) and agitated for 90 minutes. The resin was then filtered
35 off and washed with 0.7 ml of trifluoroacetic acid/water (19:1). The combined trifluoroacetic acid and water solutions were then evaporated in a vacuum centrifuge and the residue was suspended in acetonitrile/water (1:1) and freeze dried. There were obtained

16.8 mg of 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L-tyrosyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid as a white solid; MS m/e 807.4 [M+H-H₂O]⁺.

5

The starting material was prepared as follows:

i) 25 ml of isobutylene were condensed at -78°C and added to a mixture of 19.4 g (114 mmol) of 3(RS),7-dimethyl-6-octenoic
10 acid and 1 ml of concentrated sulphuric acid in 25 ml of dichloromethane. The mixture was stirred for 24 hours under a dry ice condenser. A further 20 ml of isobutylene were added and the mixture was stirred for 24 hours under a dry ice condenser. The mixture was diluted with dichloromethane, washed with
15 saturated sodium bicarbonate solution, dried over anhydrous magnesium sulphate and evaporated under a vacuum. The resulting oil was purified by chromatography on silica gel using ethyl acetate/hexane (1:9) for the elution. There were obtained
20 20.8 g of tert-butyl 3(RS),7-dimethyl-6-octenoate as a colourless oil. ¹H NMR (250 MHz, CDCl₃) δ : 0.9 (d, 3H), 1.1-1.3 (m, 3H), 1.4 (s, 9H), 1.6 (s, 3H), 1.65, (s, 3H), 1.8-2.2 (br m, 4H), 5.05, (m, 1H).

ii) 1.5 g (6.64 mmol) of tert-butyl 3(RS),7-dimethyl-6-
25 octenoate were dissolved in a mixture of 10 ml of acetone, 2 ml of water and 2 ml of glacial acetic acid. 2 g (12.6 mmol) of potassium permanganate were added and the resulting mixture was stirred at 30°C for 2 hours. 22 ml of 2M sulphuric acid and 0.8 g (11.3 mmol) of sodium nitrite were added and the organic
30 phase was separated. The aqueous phase was extracted with dichloromethane and the combined organic phases were washed with water, dried over magnesium sulphate and evaporated under a vacuum to give 1.55 g of tert-butyl 7-hydroxy-3(RS),7-dimethyl-6-oxo-octenoate as a clear oil; MS: m/e 259 [M+H]⁺.

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iii) 0.25 g (0.97 mmol) of tert-butyl 7-hydroxy-3(RS),7-dimethyl-6-oxo-octenoate was dissolved in 3 ml of diethyl ether at 0°C under a nitrogen atmosphere. 0.36 ml (1.1 mmol) of 3M

methybmagnesium bromide in diethyl ether was added dropwise and the resulting solution was stirred at 0°C for 2 hours, refluxed for 6 hours and then stirred at room temperature for 16 hours. The solution was diluted with ethyl acetate and then
5 extracted with 2M hydrochloric acid and saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulphate and evaporated under a vacuum. The resulting oil was purified by chromatography on silica gel using ethyl acetate/hexane (1:2) for the elution. There were obtained 118 mg of
10 tert-butyl 6(RS),7-dihydroxy-3(RS),6,7-trimethyl-6-octenoate as a clear oil; MS: m/e 275 [M+H]⁺.

iv) 0.64 g (2.3 mmol) of tert-butyl 6(RS),7-dihydroxy-3-(RS),6,7-trimethyl-6-octenoate was stirred in 3 ml of tetrahydro-
15 furan with 0.5 g (2.5 mmol) of dichloromethyl diisopropoxyborane at room temperature for 16 hours. The resulting mixture was evaporated and the residue was co-evaporated with toluene to give 0.86 g of tert-butyl 5-[2-(dichloromethyl)-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate as an
20 oil which was used in the next step without further purification.

v) 0.86 g (2.3 mmol) of tert-butyl 5-[2-(dichloromethyl)-4(RS), 5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate was dissolved in 5 ml of tetrahydrofuran and the
25 solution was cooled to -78°C under a nitrogen atmosphere. 2.6 ml (2.6 mmol) of 1M ethylmagnesium bromide in tetrahydrofuran were added dropwise, the resulting solution was stirred for 16 hours while slowly warming to room temperature and then diluted with ethyl acetate and extracted with 2M hydrochloric
30 acid and brine. The organic phase was dried over sodium sulphate and then evaporated under a vacuum to give 0.83 g of tert-butyl 5-[2-(1(RS)-chloropropyl)-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate as an oil which was used in the next
35 step without purification.

vi) 0.82 g (2.27 mmol) of tert-butyl 5-[2-(1(RS)-chloropropyl)-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate was dissolved in 10 ml of tetrahydrofuran and

- then cooled to -78°C under a nitrogen atmosphere. 2.3 ml (2.3 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran were added dropwise. The solution was then stirred overnight while slowly warming to room temperature. The solvent
- 5 was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration and the filtrate was cooled to 0°C . 0.52 ml (6.8 mmol) of trifluoroacetic acid was added and the solution was stirred at 0°C for 30 minutes. The solution was evaporated and the residue was
- 10 co-evaporated with toluene to give 1 g of tert-butyl 5-[2-(1(RS)-aminopropyl)-4(RS), 5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate as an oil which was used in the next step without purification.
- 15 vii) 0.5 g (1.42 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-L-leucine was dissolved in 7 ml of dichloromethane. 0.6 ml (5.7 mmol) of N-methylmorpholine was added and the solution was cooled to -10°C under a nitrogen atmosphere. 0.22 ml (1.7 mmol) of isobutyl chloroformate was added and the solution
- 20 was stirred for 7 minutes at -10°C . 1 g (2.13 mmol) of tert-butyl 5-[2-(1(RS)-aminopropyl)-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate was added and the mixture was stirred at room temperature for 16 hours, then diluted with dichloromethane and extracted with 2M hydrochloric
- 25 acid. The organic phase was extracted with 2M hydrochloric acid and saturated sodium hydrogen carbonate solution and then dried over anhydrous magnesium sulphate. After evaporation the residue was purified by chromatography on silica gel using ethyl acetate/hexane (1:2) for the elution. There was obtained 0.56 g
- 30 of tert-butyl 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate as an oil; MS: m/e 677 $[\text{M}+\text{H}]^{+}$.
- viii) 50 mg (0.074 mmol) of tert-butyl 5-[2-[1(RS)-[[N-[(9-
- 35 fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvalerate were dissolved in 1 ml of trifluoroacetic acid and 1 ml of dichloromethane. The solution was stirred at room temperature for

15 minutes and then evaporated under a vacuum. The residue was co-evaporated with toluene to give 46 mg of 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvaleric acid as
5 an oil; MS: m/e 621 [M+H]⁺.

ix) 5 g (5.25 mmol) of 4-methylbenzhydrylamine resin were swollen in dimethylformamide and excess solvent was drained from the resin. The resin was then resuspended in dimethyl-
10 formamide containing 3.4 g (5.48 mmol) of 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methylvaleric acid and 3 g (8.2 mmol) of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetra-
15 methyluronium hexafluorophosphate. Thereto there were added 3.0 ml (16.5 mmol) of diisopropylamine. The resulting mixture was agitated for 100 minutes and the resin was then drained and washed three times with dimethylformamide. The resin was then resuspended in dimethylformamide containing 5 ml (54.8 mmol) of acetic anhydride and 11.5 ml (110 mmol) of N-methylmorpho-
20 line. The mixture was agitated for 30 minutes and the resin was then drained. The resin was then resuspended in dimethylformamide containing 5 ml (54.8 mmol) of acetic anhydride and 11.5 ml (110 mmol) of N-methylmorpholine. The mixture was agitated for 30 minutes and the resin was then drained and
25 washed three times with dimethylformamide, twice with ethyl acetate, twice with dichloromethane and twice with diethyl ether and then dried under a vacuum. After drying there was obtained 6 g of 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]-amino]propyl] -4-(RS),5,5-trimethyl-1,3,2-dioxoborolan-4-yl]-
30 3(RS)-methyl-N-[α (RS)-(4-methylphenyl)benzyl]valeramide-polystyrene conjugate as a pale brown solid (0.25 mmol/g loading estimated by quantitation of dibenzofulvene at 301 nM).

Example 24

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In an analogous manner to that described in Example 5, from N2-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenyl-

- alanyl]-3-methyl-L-valyl]-3-cyclopentyl-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-alaninamide there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanyl]amino]-3-butenylboronic acid as a white solid; MS: m/e 855 [M+H-H₂O].

The starting material was prepared as follows:

- 10 i) A mixture of 1.2 g (4.67 mmol) of N-(tert-butoxycarbonyl)-3-cyclopentyl-L-alanine, 540 mg (5 mmol) of benzyl alcohol, 675 mg (5 mmol) of 1-hydroxybenzotriazole, 1.152 g (6 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.031 g (0.25 mmol) of 4-dimethylamino-
15 pyridine was stirred in 20 ml of dichloromethane for 1 hour and then a further 610 mg (5 mmol) of 4-dimethylaminopyridine were added. After 4 hours the solution was extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution, dried over anhydrous magnesium sulphate and evaporated. The oil
20 obtained was chromatographed on silica gel using ethyl acetate/petrol (1:6) for the elution to give 1.55 g of N-(tert-butoxycarbonyl)-3-cyclopentyl-L-alanine benzyl ester as a colourless oil; MS: m/e 348 [M+H].
- 25 ii) 1.54 g (4.44 mmol) of N-(tert-butoxycarbonyl)-3-cyclopentyl-L-alanine benzyl ester and 2.53 g (13.32 mmol) of 4-toluenesulphonic acid hydrate were dissolved in 20 ml of acetonitrile and the solution was left to stand at room temperature for 18 hours. The white precipitate formed was filtered off and
30 added to a mixture of 867 mg (3.75 mmol) of N-(tert-butoxycarbonyl)-3-methyl-L-valine, 557 mg (3.64 mmol) of 1-hydroxybenzotriazole, 793 mg (4.14 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 475 mg (4.13 mmol) of N-ethylmorpholine in 25 ml of dichloromethane
35 and stirred at room temperature for 18 hours. The solution was extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution and then dried over anhydrous magnesium sulphate. Evaporation and chromatography on silica gel using

ethyl acetate/petrol (1:3) for the elution gave 1.06 g of N-[N-(tert-butoxycarbonyl)-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester as an off-white foam; MS: m/e 461 [M+H].

- 5 iii) 993 mg (2.16 mmol) of N-[N-(tert-butoxycarbonyl)-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester and 1.23 g (6.47 mmol) of 4-toluenesulphonic acid hydrate were dissolved in 20 ml of acetonitrile and the solution was stirred at room temperature for 2 hours. The solvent was removed by evaporation and the residue was triturated with diethyl ether and filtered off. The solid obtained was added to a mixture of 602 mg (2.16 mmol) of N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanine, 338 mg (2.21 mmol) of 1-hydroxybenzotriazole, 576 mg (3 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 345 mg (3 mmol) of N-ethylmorpholine in 20 ml of dichloromethane and stirred at room temperature for 18 hours. The solution was extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution, then dried over anhydrous magnesium sulphate and evaporated.
- 10 Chromatography of the residue on silica gel using ethyl acetate/petrol (3:7) for the elution gave 990 mg of N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester as a white solid; MS: m/e 622 [M+H].
- 20 iv) 980 mg (1.578 mmol) of N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester and 900 mg (4.73 mmol) of 4-toluenesulphonic acid hydrate were dissolved in 16 ml of acetonitrile and the solution was stirred at room temperature for 2 hours.
- 25 The solvent was removed by evaporation and the residue was triturated with diethyl ether and filtered off. The solid obtained was added to a mixture of 671 mg (1.578 mmol) of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -glutamic acid, 247 mg (1.614 mmol) of 1-hydroxybenzotriazole, 419 mg (2.19 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 252 mg (2.19 mmol) of N-ethylmorpholine in 16 ml of dichloromethane and stirred at room temperature for 18 hours. The solution was extracted with 2M hydrochloric acid and
- 30
- 35

saturated sodium bicarbonate solution and then dried over anhydrous magnesium sulphate. Evaporation and chromatography on silica gel using methanol/dichloromethane (1:49) for the elution gave 530 mg of N-[N-[N-[O-tert-butyl-N-(9-fluorenyl-methoxycarbonyl)-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester as a white solid; MS: m/e 929 [M+H].

v) A solution of 520 mg (0.56 mmol) of N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester in 3 ml of piperidine and 12 ml of dichloromethane was stirred at room temperature for 30 minutes. The solvent was removed by evaporation and the residue was chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and then methanol/dichloromethane (1:9) for the elution. The resulting amine was added to a solution of 207 mg (0.504 mmol) of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -aspartic acid, 78 mg (0.51 mmol) of 1-hydroxybenzotriazole and 134 mg (0.7 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride in 10 ml of dichloromethane and stirred at room temperature for 18 hours. The solution was then extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution and dried over anhydrous magnesium sulphate. Evaporation, trituration with diethyl ether and filtration gave 440 mg of N-[N-[N-[N-O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester as a white solid; MS: m/e 1101 [M+H].

vi) A solution of 430 mg (0.39 mmol) of N-[N-[N-[N-O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester in 4 ml of piperidine and 16 ml of dichloromethane was stirred at room temperature for 30 minutes and then evaporated. The residue was chromatographed on silica gel using firstly ethyl acetate/petrol (1:1) and

then methanol/dichloromethane (1:9) for the elution. The amine obtained was added to a solution of 174 mg (1 mmol) of tert-butyl hydrogen succinate, 135 mg (1 mmol) of 1-hydroxy-benzotriazole and 192 mg (1 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride in 15 ml of dichloromethane and the mixture was stirred at room temperature for 18 hours, extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution and then dried over anhydrous magnesium sulphate. Evaporation and chromatography on silica gel using methanol/dichloromethane (1:24) for the elution followed by trituration with diethyl ether gave 240 mg of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester as a white solid; MS: m/e 1035 [M+H].

vii) A solution of 230 mg (0.223 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine benzyl ester in 10 ml of dimethylformamide was hydrogenated over 25 mg of 10% palladium/carbon for 3 hours. The catalyst was removed by filtration, the filtrate was evaporated and the residue was trituated with diethyl ether to give 206 mg of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine as a white solid; MS: m/e 944 [M+H].

viii) 163 mg (0.173 mmol) of N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanine were dissolved in 2 ml of dimethylformamide and 4 ml of dichloromethane. 80 mg (0.69 mmol) of N-ethylmorpholine were added and the solution was cooled to -10°C. 26 mg (0.19 mmol) of isobutyl chloroformate were added and the solution was stirred for 30 minutes at -10°C. 107 mg (0.345 mmol) of α -(RS)-allyl-4,4,5,5-tetramethyl-1,3,2-

dioxaborolane-2-methylamine trifluoroacetate in 1 ml of dichloromethane were added and the mixture was stirred at -10°C for 30 minutes and at room temperature for 3 hours. The solution was extracted with 2M hydrochloric acid and saturated sodium bicarbonate solution and then dried over anhydrous magnesium sulphate. Evaporation and chromatography on silica gel using methanol/dichloromethane (1:24) for the elution gave 54 mg of N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-alaninamide as a white solid; MS: m/e 1024 [M+H-C₆H₁₂O].

Example 25

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In an analogous manner to that described in Example 5, from N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide, MS: m/e 1037 [M+H-C₆H₁₂O], there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucyl]amino]-3-butenylboronic acid; MS: m/e 869 [M+H-H₂O].

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The starting material was prepared in an analogous manner to that described in Example 5 via the following intermediates:

i) N-[N-(tert-Butoxycarbonyl)-3-cyclohexyl-L-alanyl]-L-leucine benzyl ester; MS: m/e 475 [M+H];

ii) N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucine benzyl ester; MS: m/e 636 [M+H];

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iii) N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucine benzyl ester; MS: m/e 944 [M+H];

iv) N-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucine benzyl ester; MS: m/e 1114 [M+H];

v) N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucine benzyl ester; MS: m/e 1049 [M+H]; and

vi) N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-cyclohexyl-L-alanyl]-L-leucine; MS: m/e 958 [M+H].

Example 26

In an analogous manner to that described in Example 5, from N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl-L-leucinamide, MS: m/e 1017 [M+H-C₆H₁₂O], there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucyl]amino]-3-butenylboronic acid; MS: m/e 849 [M+H-H₂O].

The starting material was prepared in an analogous manner to that described in Example 5 via the following intermediates:

- i) N-[N-(tert-Butoxycarbonyl)-L-2-phenylglycyl]-L-leucine benzyl ester; MS: m/e 455 [M+H];
- ii) N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucine benzyl ester; MS: m/e 616 [M+H];
- iii) N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L-

α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucine benzyl ester; MS: m/e 923 [M+H];

iv) N-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucine benzyl ester; MS: m/e 1094 [M+H];

v) N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucine benzyl ester; MS: m/e 1028 [M+H]; and

vi) N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-phenylglycyl]-L-leucine; MS: m/e 938 [M+H].

Example 27

20 In an analogous manner to that described in Example 5, from N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl-L-leucinamide, MS: m/e 1023
25 [M+H-C₆H₁₂O], there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucyl]amino]-3-butenylboronic acid; MS: m/e 855 [M+H-H₂O].

30 The starting material was prepared in an analogous manner to that described in Example 5 via the following intermediates:

i) N-[N-(tert-Butoxycarbonyl)-L-2-cyclohexylglycyl]-L-leucine benzyl ester; MS: m/e 461 [M+H];

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ii) N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucine benzyl ester; MS: m/e 622 [M+H];

- iii) N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucine benzyl ester; MS: m/e 929 [M+H];
- 5 iv) N-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucine benzyl ester; MS: m/e 1100 [M+H];
- 10 v) N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucine benzyl ester; MS: m/e 1034 [M+H]; and
- 15 vi) N-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucine; MS: m/e 944 [M+H].

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Example 28

- In an analogous manner to that described in Example 5, from N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenyl-
- 25 alanyl]-3-methyl-L-valyl]-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl-L-prolinamide, MS: m/e 981 [M+H-C₆H₁₂O], there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-prolyl]amino]-3-butenyl-
- 30 boronic acid as a white solid; MS: m/e 813 [M+H-H₂O].

The starting material was prepared in an analogous manner to that described in Example 5 via the following intermediates:

- 35 i) N-[N-(tert-Butoxycarbonyl)-3-methyl-L-valyl]-L-proline benzyl ester;

- ii) N-[N-[N-(tert-butoxycarbonyl)-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-proline benzyl ester;
- iii) N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-proline benzyl ester;
- iv) N-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-
10 L-phenylalanyl]-3-methyl-L-valyl]-L-proline benzyl ester;
- v) N-[N-[N-[N-[O-tert-butyl-N-[3-(tert-butoxycarbonyl)-propionyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl-L-proline benzyl ester; MS:
15 m/e 992 [M+H]; and
- vi) N-[N-[N-[N-[O-tert-butyl-N-[3-(tert-butoxycarbonyl)-propionyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl-L-proline.

20

Example 29

In an analogous manner to that described in Example 5, from
N2-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-
25 L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-phenylalanyl]-N1-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl-L-leucinamide, MS: m/e 1031 [M+H-C₆H₁₂O], there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenyl-
30 alanyl]-L-phenylalanyl]-L-leucyl]amino]-3-butenylboronic acid as a white solid; MS: m/e 863 [M+H-H₂O].

The starting material was prepared in an analogous manner to that described in Example 5 via the following intermediates:

35

- i) N-[N-[N-[O-tert-Butyl-N-[(9-fluorenyl)methoxycarbonyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-phenylalanyl]-L-leucine benzyl ester;

ii) N-[N-[N-[N-[O-tert-butyl-N-[(9-fluorenyl)methoxy-carbonyl]-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-phenylalanyl]-L-leucine benzyl ester;

5

iii) N-[N-[N-[N-N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-phenylalanyl]-L-leucine benzyl ester; MS m/e 1042 [M+H]; and

10

iv) N-[N-[N-[N-N-[3-(tert-butoxycarbonyl)propionyl]-3-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-phenylalanyl]-L-leucine.

15

Example 30

0.04 g (0.03 mmol) of (E)-N²-[N-[N-[N-[N-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-

20 [1(S)-(dimethoxymethyl)-3-pentyl]-L-leucinamide was dissolved in 4 ml of a 1:1 solution of dichloromethane and trifluoroacetic acid containing 3 drops of water. The resulting solution was stirred at room temperature for 1 hour. After removal of the solvent by evaporation and trituration of the residue with diethyl
25 ether there was obtained 0.014 g of (E)-2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexenal; MS: m/e 845.7 [M+H]⁺.

30

The starting material was prepared as follows:

i) 25 g (347 mmol) of trans-2-buten-1-ol were dissolved in 750 ml of anhydrous diethyl ether. 7.25 ml (89.63 mmol) of anhydrous pyridine were added and the resulting solution was
35 cooled to 0°C. 88.25 ml of phosphorus tribromide were added dropwise and the mixture was stirred for 2 hours at 0°C. The reaction was quenched by pouring the solution on to ice. The organic phase was washed with saturated sodium chloride

solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation there was obtained (E)-1-bromo-2-butane which was used in the next step without purification.

5

ii) 3.86 g (168 mmol) of sodium metal were dissolved in 106 ml of anhydrous ethanol. 36.35 g (168 mmol) of diethyl acetamidomalonate dissolved in 225 ml of anhydrous ethanol were added and the mixture was heated under reflux for 10 minutes. 22.66 g (168 mmol) of (E)-1-bromo-2-butene were added dropwise at room temperature and the mixture was stirred overnight and then evaporated to dryness under a vacuum. The residue was partitioned between ethyl acetate and 0.1M hydrochloric acid. The organic phase was washed with saturated 15 sodium hydrogen carbonate solution and then with saturated sodium chloride solution and dried over anhydrous magnesium sulphate. The solvent was evaporated to give 40 g of diethyl (E)-2-acetamido-2-(2-butenyl)malonate as a colourless oil; ¹H NMR (250 MHz, CDCl₃) δ: 1.25 (t, 6H), 1.6 (d, 3H), 2.0 (s, 3H), 2.9 (d, 20 2H), 4.2 (q, 4H), 5.15 (m, 1H) 5.5 (m, 1H), 6.7 s, 1H).

iii) 39.63 g (146 mmol) of diethyl (E)-2-acetamido-2-(2-butenyl)malonate were dissolved in 200 ml of ethanol and a solution of 19.24 g (481 mmol) of sodium hydroxide in 100 ml 25 of water was added. The mixture was stirred for 2 hours at 60°C, evaporated to dryness under a vacuum and the residue was partitioned between diethyl ether and water. The aqueous phase was acidified with 2M hydrochloric acid and extracted with ethyl acetate. The organic phase was dried over magnesium sulphate 30 and the solvent was removed by evaporation under a vacuum to give 26.1 g of (E)-2-acetamido-2-(2-butenyl)malonic acid as a white solid which was used in the next step without further purification. ¹H NMR (250 MHz, MeOD) δ: 1.65 (d, 3H), 2.0 (s, 3H), 2.9 (d, 2H), 5.25 (m, 1H), 5.5 (m, 1H).

35

iv) 26.1 g (121 mmol) of (E)-2-acetamido-2-(butenyl)malonic acid were dissolved in 200 ml of toluene. 34 ml (242 mmol) of triethylamine were added and the mixture was heated under

- reflux for 1 hour. The solution was extracted with 1M hydrochloric acid and the aqueous layer was extracted with ethyl acetate. The combined organic phases were dried over magnesium sulphate and the solvent was removed under a vacuum to give
- 5 18.73 g of (E)-N-acetyl-DL-2-(2-butenyl)glycine as a white solid which was used in the next step without purification. ^1H NMR (250 Hz, MeOD) δ : 1.65 (d, 3H), 2.0 (s, 3H), 2.4 (m, 2H), 4.3 (m, 1H), 5.4 (m, 1H), 5.5 (m, 1H).
- 10 v) 9 g (52.63 mmol) of (E)-N-acetyl-DL-2-(2-butenyl)glycine were dissolved in 100 ml of water and the pH adjusted to 7.5 using ammonia solution. 0.09 g of acylase I extracted from porcine kidney, and 0.042 g (0.3 mmol) of cobalt (II) chloride were added and the mixture was stirred at 37°C overnight. A
- 15 further 0.09 g of acylase I extracted from porcine kidney was added and the pH adjusted to 7.5 using ammonia solution. The mixture was stirred at 37°C overnight and the solution was then heated at 80°C for 30 minutes and was then acidified to pH 1 using 2 M hydrochloric acid. The solvent was removed by evap-
- 20 oration under vacuum and the crude product purified by trituration using ethyl acetate to yield 4.2 g of (E)-L-2-(2-butenyl)-glycine hydrochloride. ^1H NMR (250 MHz, D₂O) δ : 1.7 (d, 3H), 2.6 (m, 2H), 4.0 (m, 1H), 5.35 (m, 1H), 5.7 (m, 1H).
- 25 vi) 2.1 g (12.69 mmol) of (E)-L-2-(2-butenyl)glycine hydrochloride were suspended in 20 ml of water and 20 ml of dioxan. 8.26 g (98.32 mmol) of sodium hydrogen carbonate and 8.15 g (37.33 mmol) of di-tert-butyl dicarbonate were added and the resulting solution was stirred for overnight. The solution was
- 30 evaporated to dryness under a vacuum and the residue was partitioned between diethyl ether and saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was acidified with 2M hydrochloric acid while partitioning in ethyl acetate. The organic phase was dried over magnesium sulphate and the
- 35 solvent was removed by evaporation to give 1.34 g of (E)-N-(tert-butoxycarbonyl)-L-2-(2-butenyl)glycine; ^1H NMR (250 MHz, CDCl₃) δ : 1.4 (s, 9H), 1.65 (d, 3H), 2.5 (m, 2H), 4.3 (m, 1H), 5.0 (m, 1H), 5.35 (m, 1H), 5.6 (m, 1H).

vii) 1.34 g (5.85 mmol) of (E)-N-(tert-butoxycarbonyl)-L-2-(2-butenyl)glycine were dissolved in 50 ml of anhydrous tetrahydrofuran and the solution was treated in sequence with 0.80 g (8.20 mmol) of N,O-dimethylhydroxylamine hydrochloride, 1.10 g (7.19 mmol) of 1-hydroxybenzotriazole monohydrate, 1.57 g (8.22 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 4 ml (22.96 mmol) of ethyldiisopropylamine. The solution obtained was stirred at room temperature overnight, then washed with saturated sodium hydrogen carbonate solution and with saturated sodium chloride solution and dried over magnesium sulphate. Removal of the solvent by evaporation yielded 1.56 g of N,O-dimethyl (E)-2(S)-(tert-butoxyformamido)-4-hexenohydroxamate as a colourless oil which was used in the next step without purification. ¹H NMR (250 MHz, CDCl₃) δ: 1.4 (s, 9H), 1.65 (d, 3H), 2.3 (m, 2H), 3.15 (s, 3H), 3.75 (s, 3H), 4.7 (m, 1H), 5.1 (d, 1H), 5.35 (m, 1H), 5.5 (m, 1H).

viii) 1.56 g (5.74 mmol) of N,O-dimethyl (E)-2(S)-(tert-butoxyformamido)-4-hexenohydroxamate were dissolved in 10 ml of anhydrous tetrahydrofuran and cooled to 0°C. 4.0 ml of a 1M solution of lithium aluminium hydride in tetrahydrofuran were added and the resulting solution was stirred for 30 minutes. The reaction was quenched by the dropwise addition of saturated potassium hydrogen sulphate solution followed by diethyl ether. The resulting two-phase system was stirred vigorously for 3 minutes. The organic phase was washed with saturated sodium hydrogen carbonate solution followed by saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation the resulting aldehyde was used without purification.

1 g (4.69 mmol) of the aldehyde was dissolved in a saturated solution of hydrogen chloride in methanol and stirred at room temperature for 2 hours. After removal of the solvent by evaporation the dimethyl acetal obtained was used without purification.

0.15 mg (0.16 mmol) of N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 0.033 g (0.22 mmol) of 1-hydroxybenzotriazole mono-hydrate, 0.047 g (0.25 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.77 g (6.69 mmol) of 4-ethylmorpholine were dissolved in 15 ml of dichloromethane. 0.05 g (0.22 mmol) of the dimethyl acetal dissolved in 5 ml of dichloromethane was added and the resulting solution was stirred at room temperature for 3 days. The mixture was washed with 5% citric acid solution followed by saturated sodium hydrogen carbonate solution and saturated sodium chloride solution and then dried over anhydrous magnesium sulphate. After evaporation of the solvent the crude product was chromatographed on silica gel using 2% methanol in dichloromethane for the elution to give 0.079 g of (E)-N²-[N-[N-[N-[N-(3-tert-butoxycarbonyl)propionyl]-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-pentyl]-L-leucinamide as a white solid foam; m/e 1027.9 [M+H-MeOH]⁺.

Example 31

0.05 g (0.04 mmol) of (Z)-N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-3-pentenyl]-L-leucinamide was dissolved in 4 ml of a 1:1 solution of dichloromethane and trifluoroacetic acid and containing 3 drops of water. The solution was stirred at room temperature for 1 hour. After removal of the solvent by evaporation the crude product was triturated using diethyl ether to afford 0.03 g of (Z)-2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L-alpha-aspartyl]-L-alpha-glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexenol as a white solid; MS: m/e 845.7 [M+H]⁺.

The starting material was prepared as follows:

i) 25 g (347 mmol) of cis-2-buten-1-ol were dissolved in 750 ml of anhydrous diethyl ether. 7.25 ml of anhydrous pyridine were added and the resulting solution cooled to 0°C. 88.25 ml of phosphorus tribromide was added dropwise and the mixture was stirred for 2 hours at 0°C. The reaction was quenched by pouring the solution onto ice. The organic phase was washed with saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation there was obtained 25.65 g of (Z)-1-bromo-2-butene; ¹H NMR (250 MHz, CDCl₃) δ: 1.65 (d, 3H), 3.9 (d, 2H), 5.6 (m, 2H).

ii) 4.37 g (190 mmol) of sodium metal were dissolved in 110 ml of anhydrous ethanol. 41.14 g (189.6 mmol) of diethyl acetamidomalonate dissolved in 270 ml of anhydrous ethanol were added and the mixture was heated under reflux for 10 minutes. 25.65 g (168 mmol) of (Z)-1-bromo-2-butene were added dropwise at room temperature and the mixture was stirred overnight, then evaporated to dryness under vacuum and the residue was partitioned between ethyl acetate and 0.1 M hydrochloric acid. The organic phase was washed with saturated sodium hydrogen carbonate solution followed by saturated sodium chloride solution and dried over anhydrous magnesium sulphate. After removal of the solvent by evaporation the crude product was chromatographed on silica gel using 66% ethyl acetate in petroleum ether as eluent to obtain 44.69 g of diethyl (Z)-2-acetamido-2-(2-butenyl)malonate as a colourless oil; ¹H NMR (250 MHz, CDCl₃) δ: 1.2 (t, 6H), 1.6 (d, 3H), 2.0 (s, 3H), 3.1 (d, 2H), 4.2 (q, 4H), 5.1(m, 1H), 5.6 (m, 1H), 6.7 (s, 1H).

iii) 44.69 g (165 mmol) of diethyl (Z)-2-acetamido-2-(2-butenyl)malonate were dissolved in 230 ml of ethanol and a solution of 21.69 g (542 mmol) of sodium hydroxide in water was added. The mixture was stirred for 2 hours at 60°C, evaporated to dryness under a vacuum and the residue was partitioned between diethyl ether and water. The aqueous phase was acidified using 2M hydrochloric acid and extracted with ethyl acetate. The organic phase was dried over magnesium sulphate and the solvent removed by evaporation in a vacuum to give 33.5 g of (Z)-2-acetamido-2-(2-butenyl)malonic acid as a

white solid; ^1H NMR (250 MHz, MeOD) δ : 1.6 (d, 3H), 2.0 (s, 3H), 2.85 (d, 2H), 5.25 (m, 1H), 5.6 (m, 1H).

- iv) 16.82 g (78.23 mmol) of (Z)-2-acetamido-2-(2-butenyl)-malonic acid were dissolved in 100 ml of toluene. 34 ml (242 mmol) of triethylamine were added and the mixture was heated under reflux for 1 h, then washed with 1M hydrochloric acid and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over magnesium sulphate and the solvent was removed under a vacuum to give 9.4 g of (Z)-N-acetyl-DL-2-(2-butenyl)glycine as a white solid; ^1H NMR (250 MHz, MeOD) δ : 1.6 (d, 3H), 2.0 (s, 3H), 2.5 (m, 2H), 4.4 (m, 1H), 5.4 (m, 1H), 5.6 (m, 1H).
- v) 9.4 g (54.97 mmol) of (Z)-N-acetyl-DL-2-(2-butenyl)glycine were dissolved in 100 ml of water and the pH was adjusted to 7.8 with ammonia solution. 0.09 g of acylase I extracted from porcine kidney, and 0.042 g (0.3 mmol) of cobalt (II) chloride were added and the resulting reaction mixture was stirred at 37°C overnight. The pH was adjusted to 7.8 using ammonia solution. The mixture was stirred at 37°C overnight and was then heated at 80°C for 30 minutes and was then acidified to pH 1 using 2M hydrochloric acid. The solution was acidified to pH 1 using 2M hydrochloric acid and then heated at 80°C for 30 minutes. The solvent was removed by evaporation under a vacuum and the crude product obtained purified by trituration using ethyl acetate to yield 5.86 g of (Z)-L-2-(2-butenyl)glycine hydrochloride; ^1H NMR (250 MHz, D_2O) δ : 1.6 (d, 3H), 2.7 (t, 2H), 4.1 (t, 1H), 5.3 (m, 1H), 5.8 (m, 1H).
- vi) 2.9 g (17.52 mmol) of (Z)-L-2-(2-butenyl)glycine hydrochloride were suspended in 25 ml of water and 25 ml of dioxan. 11.4 g (136 mmol) of sodium hydrogencarbonate and 8.49 g (38.94 mmol) of di-tert-butyl dicarbonate were added and the resulting solution was stirred for 48 hours. The solution was evaporated to dryness under a vacuum and the residue was partitioned between diethyl ether and saturated aqueous sodium hydrogen carbonate solution. The aqueous phase was acidified

using 2M hydrochloric acid whilst being partitioned with ethyl acetate. The organic phase was dried over magnesium sulphate and the solvent removed by evaporation to give 2.26 g of (Z)-N-(tert-butoxycarbonyl)-L-2-(2-butenyl)glycine; ¹H NMR (250 MHz, CDCl₃) δ: 1.4 (s, 9H), 1.6 (d, 3H), 2.6 (m, 2H), 4.4 (m, 1H), 5.05 (m, 1H), 5.3 (m, 1H), 5.6 (m, 1H).

vii) 2.26 g (9.87 mmol) of (Z)-N-(tert-butoxycarbonyl)-L-2-(2-butenyl)glycine were dissolved in 50 ml of anhydrous tetrahydrofuran. 1.15 g (11.79 mmol) of N,O-dimethylhydroxylamine hydrochloride, 1.6 g (10.46 mmol) of 1-hydroxybenzotriazole monohydrate, 2.27 g (11.88 mmol) of 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride and 5.8 ml of ethyldiisopropylamine were added and the resulting solution was stirred at room temperature overnight. The solution was washed with saturated sodium hydrogen carbonate solution followed by saturated sodium chloride solution and then dried over anhydrous magnesium sulphate. Removal of the solvent by evaporation yielded 2.46 g of N,O-dimethyl (Z)-2(S)-(tert-butoxyformamido)-4-hexenohydroxamate as a colourless oil; ¹H NMR (250 MHz, CDCl₃) δ: 1.4 (s, 9H), 1.6 (d, 3H), 2.35 (m, 1H), 2.5 (m, 1H), 3.2 (s, 3H), 3.75 (s, 3H), 4.7 (m, 1H), 5.2 (d, 1H), 5.35 (m, 1H), 5.6 (m, 1H).

viii) 1.01 g (3.71 mmol) of N,O-dimethyl (Z)-2(S)-(tert-butoxyformamido)-4-hexenohydroxamate were dissolved in 10 ml of anhydrous tetrahydrofuran and cooled to 0°C. 2.6 ml of a 1M solution of lithium aluminium hydride in tetrahydrofuran were added and the resulting solution was stirred for 30 minutes. The reaction was quenched by the dropwise addition 15 ml of saturated potassium hydrogen sulphate solution followed by 30 ml of diethyl ether. The resulting two-phase system was stirred vigorously for one hour. The organic phase was washed with saturated sodium hydrogen carbonate solution followed by saturated sodium chloride solution and dried over magnesium sulphate. After removal of the solvent by evaporation the aldehyde was used without further purification. 0.79 g (3.71 mmol) of the aldehyde was dissolved in a saturated solution of hydrogen chloride in 10 ml of methanol and stirred at room temperature for 2 hours. After removal of the solvent by evaporation the

dimethylacetal obtained was used without purification

0.15 g (0.16 mmol) of N-[N-[N-[N-[3-(tert-butoxy-carbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L-
5 α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine, 0.033 g (0.22 mmol) of 1-hydroxybenzotriazole mono hydrate, 0.047 g (0.25 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and 0.77 g (6.69 mmol) of 4-ethylmorpholine were dissolved in 15 ml of dichloromethane.
10 0.05 g (0.22 mmol) of the foregoing dimethyl acetal dissolved in 5 ml of dichloromethane was added and the resulting solution was stirred at room temperature for 3 days. The solution was washed with 5% citric acid solution followed by saturated sodium hydrogen carbonate solution and saturated sodium chloride
15 solution and then dried over magnesium sulphate. After removal of the solvent by evaporation the crude product was chromatographed on silica gel using 2% methanol in dichloromethane for the elution to give 0.092 g of (Z)-N₂-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-
20 valyl]-N₁-[1(S)-(dimethoxymethyl)-3-pentenyl]-L-leucinamide, as a white solid foam; MS: m/e 1027.9 [M + H - MeOH]⁺.

Example 32

25

In an analogous manner to that described in Example 10, but using N-(tert-butoxycarbonyl)-3-(2-furyl)-L-alanine in place of N-(tert-butoxycarbonyl)-L-allylglycine there was obtained 2(RS)-
[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -
30 glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(2-furyl)propionaldehyde; MS: m/e 871.4 [M+H]⁺.

35

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

i) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-2-(2-furyl)-propionohydroxamate; ¹H NMR (250 MHz, CDCl₃) δ : 1.4 (s, 9H), 3.0 (m, 2H), 3.2 (s, 3H), 3.7 (s, 3H), 4.9 (m, 1H), 5.3 (br. d, 1H), 6.1 (br. s, 1H), 6.3 (br. s, 1H), 7.3 (br. s, 1H).

- ii) N²-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[2-(2-furyl)-1(S)-(dimethoxymethyl)ethyl]-L-leucinamide; used directly in the next step.

Example 33

- 10 In an analogous manner to that described in Example 10, but using N-(tert-butoxycarbonyl)-L-norvaline in place of N-(tert-butoxycarbonyl)-L-allylglycine there was obtained 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-
15 valeraldehyde; MS: m/e 833.4 [M+H]⁺.

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

- 20 N,O-Dimethyl 2(S)-(tert-butoxyformamido)valerohydroxamate; ¹H NMR (250 MHz, CDCl₃) δ : 0.8 (m, 3H), 1.2-1.7 (m, 4H), 1.4 (s, 9H), 3.1 (s, 3H), 3.7 (s, 3H), 4.6 (m, 1H), 5.1 (br. d, 1H).

- N²-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-butyl]-L-leucinamide; MS: m/e 1069.6 [M+Na]⁺.

Example 34

- 30 In an analogous manner to that described in Example 10, but using N-(tert-butoxycarbonyl)-L-butylglycine in place of N-(tert-butoxycarbonyl)-L-allylglycine there was obtained 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-
35 hexanal; MS: m/e 847.4 [M+H]⁺.

The starting material was prepared in an analogous manner to that described in example 10 via the following intermediates:

- 5 i) N,O-Dimethyl 2(S)-(tert-butoxyformamido)hexanohydroxamate; ^1H NMR (250 MHz, CDCl_3) δ : 0.9 (m, 3H), 1.2-1.8 (m, 6H), 1.4 (s, 9H), 3.2 (s, 3H), 3.7 (s, 3H), 4.6 (m, 1H), 5.1 (br. d, 1H).
- 10 ii) N2-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(S)-(dimethoxymethyl)-pentyl]-L-leucinamide; MS: m/e 1083 $[\text{M} + \text{Na}]^+$.

Example 35

- 15 In an analogous manner to that described in Example 10, but using DL-hexylglycine in place of L-allylglycine hydrochloride there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]octanal; MS: m/e 875.5 $[\text{M} + \text{H}]^+$.

20

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

- 25 i) 2(RS)-(tert-Butoxyformamido)octanoic acid; ^1H NMR (250 MHz, CDCl_3) δ : 0.9 (m, 3H), 1.2-1.9 (m, 10H), 1.4 (s, 9H), 4.3 (m, 1H), 5.0 (br. d, 1H)
- 30 ii) N,O-Dimethyl 2(RS)-(tert-butoxyformamido)octanohydroxamate; ^1H NMR (250 MHz, CDCl_3) δ : 0.9 (m, 3H), 1.2-1.8 (m, 10H), 1.4 (s, 9H), 3.2 (s, 3H), 3.7 (s, 3H) 4.6 (m, 1H), 5.1 (br. d, 1H)
- 35 iii) N2-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N1-[1(RS)-(dimethoxymethyl)-heptyl]-L-leucinamide; MS: m/e 1111.6 $[\text{M} + \text{Na}]^+$.

Example 36

In an analogous manner to that described in Example 10, but using 2(S)-amino-5-methylhexanoic acid in place of L-allyl-glycine hydrochloride there was obtained 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-methyl-hexanal; MS: m/e 861.3 [M+H]⁺

10 The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

- i) 2(S)-(tert-Butoxyformamido)-5-methylhexanoic acid; ¹H NMR (250 MHz, CDCl₃) δ : 0.9 (d, 6H), 1.2 (m, 2H), 1.4 (s, 9H), 1.5 (m, 1H), 1.7 (m, 1H), 1.9 (m, 1H), 4.3 (m, 1H), 4.9 (br. d, 1H).
- 15 ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-5-methylhexanohydroxamate; ¹H NMR (250 MHz, CDCl₃) δ : 0.85 (d, 3H), 0.9 (d, 3H), 1.2 (m, 2H), 1.4 (s, 9H), 1.4-1.8 (m, 3H), 3.2 (s, 3H), 3.8 (s, 20 3H), 4.6 (m, 1H), 5.1 (br. d, 1H).
- iii) N²-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-4-methylpentyl]-L-leucinamide; MS: m/e 1043.8 [M+H-MeOH]⁺.
- 25

Example 37

In an analogous manner to that described in Example 10, but using 2(S)-amino-5-hexenoic acid in place of L-allylglycine hydrochloride there was obtained 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-hexenal; MS: m/e 845.3 [M+H]⁺.

35

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

- i) 2(S)-tert-Butoxyformamido)-5-hexenoic acid; ¹H NMR (250 MHz, CDCl₃) δ: 1.4 (s, 9H), 1.8 (m, 1H), 1.95 (m, 1H), 2.2 (m, 2H), 4.3 (m, 1H), 5.0 (m, 3H), 5.8 (m, 1H).
- 5 ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-5-hexeno-hydroxamate; ¹H NMR (250 MHz, CDCl₃) δ: 1.4(s, 9H), 1.6-1.8 (m, 2H), 2.1 (m, 2H), 3.2 (s, 3H), 3.7 (s, 3H) 4.7 (m, 1H), 5.0 (m, 3H), 5.8 (m, 1H).
- 10 iii) N²-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-α-aspartyl]-O-tert-butyl-L-α-glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-4-pentenyl]-L-leucinamide; MS: m/e 1081.6 [M + Na]⁺.

15

Example 38

In an analogous manner to that described in Example 10, but using 2(S)-amino-5-hexynoic acid in place of L-allylglycine hydrochloride there was obtained 2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L-α-aspartyl]-L-α-glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-hexynal; MS: m/e 843.3 [M+H]⁺.

20

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

25

- i) 2(S)-(tert-Butoxyformamido)-5-hexynoic acid; ¹H NMR (250 MHz, MeOD) δ: 1.4 (s, 9H), 1.8 (m, 1H), 2.0 (m, 1H), 2.3 (m, 3H), 4.2 (m, 1H).
- 30 ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-5-hexyno-hydroxamate; ¹H NMR (250 MHz, MeOD) δ: 1.4 (s, 9H), 1.7 (m, 1H), 1.9 (m, 1H), 2.3 (m, 3H), 3.2 (s, 3H), 3.8 (s, 3H), 4.7 (m, 1H)
- 35 iii) N²-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-α-aspartyl]-O-tert-butyl-L-α-glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)-4-pentynyl]-L-leucinamide; MS: m/e 1079.5 [M+Na]⁺

Example 39

In an analogous manner to that described in Example 10, but
5 using N-(tert-butoxycarbonyl)-L-methionine in place of N-(tert-butoxycarbonyl)-L-allylglycine there was obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-(methylthio)butyraldehyde; MS: m/e 865.3 [M+H]⁺.

10

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

i) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-4-(methyl-
15 thio)butyroxamate; ¹H NMR (250 MHz, CDCl₃) δ : 1.4 (s, 9H), 1.75 (m, 1H), 2.0 (m, 1H), 2.05 (s, 3H), 2.5 (m, 2H), 3.2 (s, 3H), 3.75 (s, 3H), 4.7 (m, 1H), 5.2 (m, 1H).

ii) N₂-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N₁-[1(S)-(dimethoxymethyl)-2-(methylthio)propyl]-L-leucinamide; MS: m/e 1047.5 [M+H-MeOH]⁺.

Example 40

25

In an analogous manner to that described in Example 10, but
using S-(3-phenylpropyl)-L-cysteine in place of L-allylglycine
hydrochloride there was obtained 2(R)-[[N-[N-[N-[N-[N-(3-
carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-
30 phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-2-[3-(phenyl)propylthio]propionaldehyde; MS: m/e 955.4 [M+H]⁺.

The starting material was prepared in an analogous manner to that described in Example 10 via the following intermediates:

35

i) N-(tert-Butoxycarbonyl)-S-(3-phenylpropyl)-L-cysteine;
¹H NMR (250 MHz, CDCl₃) δ : 1.4 (s, 9H), 1.9 (m, 2H), 2.55 (t, 2H),
2.7 (t, 2H), 3.0 (m, 2H), 4.5 (m, 1H), 5.4 (m, 1H), 7.2 (m, 5H).

ii) N,O-Dimethyl 2(S)-(tert-butoxyformamido)-3-(3-phenyl-propylthio)propionohydroxamate; ¹H NMR (250 MHz, CDCl₃) δ: 1.4 (s, 9H), 1.9 (m, 2H), 2.5 (t, 2H), 2.7 (t, 2H), 2.8 (m, 2H), 3.2 (s, 3H),
5 3.7 (s, 3H), 4.8 (m, 1H), 5.3 (m, 1H), 7.2 (m, 5H).

iii) N²-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-α-aspartyl]-O-tert-butyl-L-α-glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(R)-(dimethoxymethyl)-2-(3-phenylpropylthio)ethyl]-L-leucinamide; MS: m/e 1191.8
10 [M+Na]⁺.

Example 41

15 In an analogous manner to that described in Example 1, but using N,O-Dimethyl 2(S)-(tert-butoxyformamido)hexanohydroxamate in place of N,O-Dimethyl 2(S)-(tert-butoxyformamido)-butyrohdroxamate and N-(9-fluorenylmethoxycarbonyl)-D-valine in place of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L-α-glutamic acid there was obtained 2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L-α-aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]hexanal; MS: m/e 817.4
20 [M+H]⁺.

25 The starting material was prepared in an analogous manner to that described in Example 1 via the following intermediates:

i) N-[N-[N-[N-[(9-Fluorenyl)methoxycarbonyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl
30 ester; MS: m/e 817.4 [M+H]⁺.

ii) N-[N-[N-[N-[(9-Fluorenyl)methoxycarbonyl]-O-tert-butyl-L-α-aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester; MS: m/e 988.4 [M+H]⁺.
35

iii) N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-α-aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester; MS: m/e 922.5 [M+H]⁺.

iv) N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine; MS: m/e 832.5 [M+H]⁺.

- 5 v) N²-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)pentyl]-L-leucinamide; MS: m/e 997.5 [M+Na]⁺.

10

Example 42

In an analogous manner to that described in Example 1, but using N,O-dimethyl 2(S)-(tert-butoxyformamido)hexanohydroxamate in place of N,O-dimethyl 2(S)-(tert-butoxyformamido)-
15 butyrohdroxamate, using N-(9-fluorenylmethoxycarbonyl)-D-valine in place of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -glutamic acid and using O-tert-butyl-N-[(9-fluorenyl)-methoxycarbonyl]-L-serine in place of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -aspartic acid there was obtained
20 2(S)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L-seryl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-hexanal; MS: m/e 789.3 [M+H]⁺.

The starting material was prepared in an analogous manner
25 to that described in Example 1 via the following intermediates:

- i) N-[N-[N-[N-[N-[(9-Fluorenyl)methoxycarbonyl]-O-tert-butyl-L-seryl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester; MS: m/e 960.4 [M+H]⁺.
30
- ii) N-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-seryl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester; MS: m/e 894.5 [M+H]⁺.
- 35 iii) N-[N-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-seryl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine; MS: m/e 804.4 [M+H]⁺.

iv) N²-[N-[N-[N-[3-(tert-Butoxycarbonyl)propionyl]-O-tert-butyl-L-seryl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)pentyl]-L-leucinamide; MS: m/e 969.7 [M+Na]⁺.

5

Example 43

In an analogous manner to that described in Example 1, but using N,O-dimethyl 2(S)-(tert-butoxyformamido)hexanohydroxamate in place of N,O-dimethyl 2(S)-(tert-butoxyformamido)-butyrylhydroxamate using N-(9-fluorenylmethoxycarbonyl)-D-valine in place of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -glutamic acid, using O-tert-butyl-N-[(9-fluorenyl)methoxycarbonyl]-L-serine in place of N-(9-fluorenylmethoxycarbonyl)-O-tert-butyl-L- α -aspartic acid and using acetic anhydride in place of tert-butyl hydrogen succinate there was obtained 2(S)-[[N-[N-[N-[N-(N-acetyl-L-seryl)-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]hexanal; MS: m/e 731.3 [M+H]⁺.

20

The starting material was prepared in an analogous manner to that described in Example 1 via the following intermediates:

- i) N-[N-[N-[N-(N-acetyl-O-tert-butyl-L-seryl)-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine; MS: m/e 690.4 [M+H]⁺.
- ii) N²-[N-[N-[N-(N-acetyl-O-tert-butyl-L-seryl)-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(S)-(dimethoxymethyl)pentyl]-L-leucinamide; MS: m/e 833.5 [M+H]⁺.

30

The reaction with acetic anhydride was carried out as follows:

- 0.5ml of N-ethylmorpholine and 0.37 ml of acetic anhydride were added in sequence to a solution of 1.95 g of N-[N-[N-(O-tert-butyl-L-seryl)-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester in 70 ml of anhydrous

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dichloromethane. The mixture was stirred at room temperature for 1 hour and was then washed in sequence with 5% aqueous citric acid solution, saturated aqueous sodium bicarbonate solution and saturated brine. The organic phase was dried over
5 anhydrous magnesium sulphate and evaporated. Chromatography of the residue on silica using 5% methanol in dichloromethane for the elution gave afforded 1.45 g of N-[N-[N-(N-acetyl-O-tert-butyl-L-seryl)-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine benzyl ester; MS: m/e 780.6 [M+H]⁺.

10

Example 44

59 mg (0.058 mmol) of N1-[4-bromo-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-N2-[N-[N-[N-(3-
15 carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucinamide were dissolved in 3 ml of trifluoroacetic acid and 3 ml of dichloromethane. 5 drops of water were added and the solution was stirred at room temperature for 3 hours. The solution was diluted with
20 toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried and then redissolved in 5 ml of trifluoroacetic acid and 5 ml of dichloromethane. The solution was stirred at room temperature for 3 hours and then diluted with toluene and evaporated. The
25 residue was triturated with diethyl ether and the solid obtained was filtered off and dried to give 30 mg of 4-bromo-1(RS)-[[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butylboronic acid in the form of a solid; MS: m/e
30 911.3 [M+H-H₂O]⁺.

The starting material was prepared as follows:

i) 1.7 ml (1.7 mmol) of 1M lithium bis(trimethylsilyl)amide
35 in tetrahydrofuran were added dropwise to a solution of 0.5 g (1.7 mmol) of 2-(4-bromo-1(RS)-chlorobutyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (prepared according to EP-A-O 293 881) in 5 ml of tetrahydrofuran under nitrogen at -78°C.

The solution was then stirred overnight at room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration and the solvent was removed by evaporation to give 0.63 g of product which was immediately redissolved in diethyl ether and cooled to 0°C. 0.34 ml (5.0 mmol) of trifluoroacetic acid was added and the solution was stirred at 0°C for 30 minutes. The solution was evaporated and the residue was evaporated with toluene to give 0.58 g of α -(RS)-3-bromopropyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate (1:1) as a brown oil which was used in the next step without purification.

ii) 0.20 g (0.22 mmol) of N-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 6 ml of dichloromethane. 0.2 ml (1.52 mmol) of N-methylmorpholine was added and the solution was cooled to -10°C under a nitrogen atmosphere. 44 mg (0.3 mmol) of isobutyl chloroformate were added and the solution was stirred for 15 minutes at -10°C. 0.3 g (0.66 mmol) of α -(RS)-3-bromopropyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate (1:1) was added and the mixture was stirred at room temperature for 5 hours. Dichloromethane was added and the solution was extracted with 2M hydrochloric acid and water and then dried over anhydrous sodium sulphate. After evaporation there was obtained 0.122 g of N₂-[N-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-methyl-L-valyl]-N₁-[4-bromo-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide in the form of a solid; MS: m/e 1079.5 [M+H-100]⁺.

iii) 115 mg (0.098 mmol) of N₂-[N-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N₁-[4-bromo-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide were dissolved in 3 ml of trifluoroacetic

acid and 3 ml of dichloromethane. 5 drops of water were added and the solution was stirred at room temperature for 3 hours. The solution was diluted with toluene and evaporated. The residue was triturated with ether and the resulting solid was
5 filtered off and dried to give 72 mg of N1-[4-bromo-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-N2-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-methyl-valyl]-L-leucinamide as a white solid; MS: m/e 911.3 [M+H-100]⁺

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Example 45

In an analogous manner to that described in Example 23, but replacing N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L-
15 tyrosine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L-aspartic acid and replacing N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valine with N-[(9-fluorenyl)methoxycarbonyl]-L-2-cyclohexylglycine there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-
20 phenylalanyl]-L-2-cyclohexylglycyl]-L-leucyl]amino]propylboronic acid as a white solid; MS: m/e 843.4 [M+H-H₂O]⁺.

Example 46

25 In an analogous manner to that described in Example 23, but replacing N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L-tyrosine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L-aspartic acid and replacing N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanine with N-[(9-fluorenyl)methoxycarbonyl]-
30 L-2-cyclohexylglycine there was obtained 1(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-L-2-cyclohexylglycyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid as a white solid; MS: m/e 795.5 [M+H-H₂O]⁺.

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Example 47

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-

fluorenyl)methoxycarbonyl]-3-cyclohexyl-L-alanine there was
obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -
aspartyl]-3-cyclohexyl-L-alanyl]-2-methyl-L-phenylalanyl]-3-
methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as
5 a white solid; MS m/e 897.6 [M+H].

Example 48

In an analogous manner to Example 4, by replacing N-[(9-
10 fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-
fluorenyl)methoxycarbonyl]-D-valine and replacing N-[(9-
fluorenyl)methoxycarbonyl]-O-t-butyl-L- α -aspartic acid with N-
[(9-fluorenyl)methoxycarbonyl]-O-t-butyl-L-serine there was
obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L-seryl]-D-
15 valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]-
amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e
815.5 [M+H].

Example 49

20 In an analogous manner to Example 4, by replacing N-[(9-
fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with [(9-
fluorenyl)methoxycarbonyl]-D-norleucine there was obtained
2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-
25 norleucyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-
leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS:
m/e 857.4 [M+H].

Example 50

30 In an analogous manner to Example 4, by replacing N-[(9-
fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-
fluorenyl)methoxycarbonyl]-D-norvaline there was obtained
2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-
35 norvalyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]-
amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e
843.4 [M+H].

Example 51

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-D-2-cyclohexylglycine there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-2-cyclohexylglycyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid, MS: m/e 897.4 [M+H].

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Example 52

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-4-nitro-D-phenylalanine there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-4-nitro-D-phenylalanyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 936.3 [M+H].

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Example 53

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valine with N-[(9-fluorenyl)methoxycarbonyl]-L-2-cyclohexylglycine and by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamic acid there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 899.5 [M+H].

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Example 54

In an analogous manner to Example 4, by replacing N-[(9-methoxycarbonyl]-3-(2-methylphenyl)-L-alanine with N-[(9-methoxycarbonyl]-L-2-cyclohexylglycine and by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-

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fluorenyl)methoxycarbonyl]-O-t-butyl-L- α -glutamic acid there was obtained 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-L-2-cyclohexylglycyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 851.4 [M+H].

Example 55

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-t-butyl-L- α -glutamic acid and by replacing tert-butyl hydrogen succinate with 3-acetamidobenzoic acid there was obtained 2(RS)-[[N-[N-[N-[N-[N-(3-acetamidobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 934.4 [M+H].

Example 56

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-t-butyl-L- α -glutamic acid and by replacing tert-butyl hydrogen succinate with 4-acetamido-3-nitrobenzoic acid there was obtained 2(RS)-[[N-[N-[N-[N-[N-(4-acetamido-3-nitrobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 979.4 [M+H].

Example 57

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamic acid and by replacing tert-butyl hydrogen succinate with 4-acetamidobenzoic acid there was obtained 2(RS)-[[N-[N-[N-[N-[N-(4-acetamidobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 934.4 [M+H].

Example 58

In an analogous manner to Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-L- α -glutamic acid and by replacing tert-butyl hydrogen succinate with 3,5-dichlorobenzoic acid there was obtained 2(RS)-[[N-[N-[N-[N-(3,5-dichlorobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 945.3 [M+H].

Example 59

0.78 g of 0.235 mmol/g 5-[2-[1(RS)-[[N-[(9-fluorenyl)methoxycarbonyl]-L-leucyl]amino]propyl]-4(RS),5,5-trimethyl-1,3,2-dioxaborolan-4-yl]-3(RS)-methyl-N-[α (RS)-(4-methylphenyl)benzyl]valeramide-polystyrene conjugate was swollen in dimethylformamide for 20 minutes and then suspended and agitated in dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained and then resuspended in and agitated with dimethylformamide/ piperidine (4:1) for a further five minutes. The resin was then drained and washed five times with dimethylformamide.

The resin was then suspended in a solution of 0.4 g, 1.08 mmol of N-[(9-fluorenyl)methoxycarbonyl]-3-methyl-L-valine in dimethylformamide and then a mixture of 0.42 g (1.08 mmol) 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.25 ml (2.2 mmol) of N-methylmorpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed five times with dimethylformamide.

The resin was resuspended in and agitated with dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained, resuspended in and agitated with dimethylformamide/ piperidine (4:1) for a further 5 minutes. Then the resin was

drained and washed five times with dimethyl formamide.

The resin was then suspended in a solution of 0.44 g (1.08 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-3-(2-methyl-phenyl)-L-alanine in dimethylformamide and then a mixture of 0.42g 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.25 ml (2.2 mmol) of N-methylmorpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed five times with dimethylformamide.

The resin was resuspended in and agitated with dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained, resuspended in and agitated with dimethylformamide/piperidine (4:1) for a further 5 minutes. Then the resin was drained and washed five times with dimethyl formamide.

The resin was then suspended in a solution of 0.37 g (1.08 mmol) of N-[(9-fluorenyl)methoxycarbonyl]-D-valine in dimethylformamide and then a mixture of 0.42 g of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.25 ml (2.2 mmol) of N-methylmorpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed five times with dimethylformamide.

The resin was resuspended in and agitated with 0.7 ml of dimethylformamide/piperidine (4:1). After 5 minutes the resin was drained, resuspended in and agitated with dimethylformamide/piperidine (4:1) for a further 5 minutes. Then, the resin was drained and washed five times with 1 ml of dimethylformamide.

98 mg of this resin were then suspended in a solution of 0.06 g (0.19 mmol) of N-(benzyloxycarbonyl)-O-tert-butyl-L- α -aspartic acid in dimethylformamide and then a mixture of 0.06 g (0.19 mmol) of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate and 0.1 ml (0.88 mmol) of N-methyl-

morpholine dissolved in dimethylformamide was added. After agitating for 40 minutes the resin was drained and washed three times with dimethylformamide, three times with ethyl acetate and three times with dichloromethane.

5

1 ml of dichloromethane was added to the resin which was then treated with 3 ml of a 9:1 mixture of trifluoroacetic acid and water and then agitated for 30 minutes. The resin was then filtered off and washed with dichloromethane. The filtrate and
10 washings were combined and evaporated and then co-evaporated with toluene. The residue was triturated with diethyl ether and dried. There were obtained 12 mg of 1(RS)-[[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid; MS:
15 m/e 821.4 [M+H-H₂O]⁺.

Example 60

200 mg (0.18 mmol) of N²-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[4-fluoro-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide were dissolved in 4.75 ml of trifluoroacetic acid and 0.25 ml of water. 2 ml of dichloromethane were
25 added and the solution was stirred at room temperature for 3 hours. The solution was diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried to give 95 mg of 4-fluoro-1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]-amino]butylboronic acid; MS: m/e 849.4 [M+H-H₂O]⁺
30

The starting material was prepared as follows:

35 i) 2.5 ml (25 mmol) of borane-dimethyl sulphide (1:1) complex were dissolved in 50 ml of dimethoxyethane and the solution was cooled to 0°C under nitrogen. 5.3 ml (52.5 mmol) of cyclohexene were then added. The solution was stirred at 0°C

for 15 minutes, then at room temperature for 1 hour and then cooled to -10°C . 1.6 g (27 mmol) of 3-fluoropropene were condensed and then added to the foregoing solution which was then stirred at room temperature under a dry ice condenser.

- 5 After 1 hour the condenser was removed and stirring was continued for a further 1 hour. 3.9 g (52 mmol) of trimethylamine N-oxide were added and the solution was stirred for 1 hour. 3.1 g (26.3 mmol) of 2,3-dimethyl-2,3-butanediol were added and the solution was stirred for 16 hours. The solution
10 was evaporated and the residue was distilled. The distillate boiling at $35\text{--}65^{\circ}\text{C}/1\text{mm Hg}$ was collected and purified by chromatography on silica gel using diethyl ether/ hexane (1:9) for the elution to give 1.67 g of 4,4,5,5-tetramethyl-2-(3-fluoropropyl)-1,3,2-dioxaborolane as a colourless oil; ^1H NMR (250 MHz, CDCl_3) δ : 0.75-0.85 (m, 2H), 1.25 (s, 12H), 1.7-1.9 (m, 2H), 4.28 (t, 1H), 4.48 (t, 1H).

- ii) 1.3 ml (8.8 mmol) of diisopropylamine and 5.5 ml (8.8 mmol) of butyllithium in hexane were added to 7 ml of
20 tetrahydrofuran at -78°C . The cooled solution was added to a solution of 1.65 g (8.8 mmol) of 4,4,5,5-tetramethyl-2-(3-fluoropropyl)-1,3,2-dioxaborolane in 0.7 ml of dichloromethane, 15 ml of cyclohexane and 8 ml of tetrahydrofuran at -20°C under nitrogen. The solution was then stirred for 16 hours while slowly
25 warming to room temperature. The solution was partitioned between 2M hydrochloric acid, brine and ethyl acetate, and the aqueous layer was extracted with ethyl acetate. The organic extracts were combined, washed with brine and dried over sodium sulphate. After evaporation the residue was purified by
30 chromatography on silica gel using diethyl ether/hexane (1:9) for the elution to give 1.0 g of 2-(4-fluoro-1(RS)-chlorobutyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane as a colourless oil; ^1H NMR (250 MHz, CDCl_3) δ : 0.75-0.85 (m, 2H), 1.3 (s, 12H), 1.9-2.1 (m, 2H), 3.45 (m, 1H) 4.35 (m, 1H), 4.55 (m, 1H).

35

- iii) 4.2 ml (4.2 mmol) of 1M lithium bis(trimethylsilyl)amide in tetrahydrofuran were added dropwise to a solution of 1.0 g (4.2 mmol) of 2-(4-fluoro-1(RS)-chlorobutyl)-4,4,5,5-tetra-

5 methyl-1,3,2-dioxaborolane in 7 ml of tetrahydrofuran under nitrogen at -78°C. The solution was then stirred overnight at room temperature. The solvent was removed by evaporation and the residue was taken up in diethyl ether. Insoluble material was removed by filtration and the solvent was removed by evaporation to give 1.53 g of material which was immediately redissolved in 7 ml of diethyl ether and cooled to 0°C. 0.95 ml (12.6 mmol) of trifluoroacetic acid was added and the solution was stirred at 0°C for 30 minutes. The solution was evaporated and the residue was evaporated with toluene to give 1.36 g of α -(RS)-3-fluoropropyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate (1:1) as a brown oil which was used in the next step without further purification.

15 iv) 0.20 g (0.22 mmol) N-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and 4 ml of dichloromethane. 0.2 ml (1.52 mmol) of N-methylmorpholine was added and the solution was cooled to -10°C under a nitrogen atmosphere. 40 mg (0.27 mmol) of isobutyl chloroformate were added and the solution was stirred for 10 minutes at -10°C. 0.2 g (0.44 mmol) of α -(RS)-3-fluoropropyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane-2-methylamine trifluoroacetate (1:1) was added and the mixture was stirred at room temperature for 16 hours. Dichloromethane was added and the solution was washed with 2M hydrochloric acid and water and then dried over anhydrous sodium sulphate. After evaporation there was obtained 0.21 g of N₂-[N-[N-[N-(tert-butoxycarbonyl)-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N₁-[4-fluoro-1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)butyl]-L-leucinamide in the form of a solid; MS: m/e 1017.3 [M+H-100]⁺.

35

Example 61

In an analogous manner to that described in Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-

alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -glutamic acid and by replacing N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanine with N-[(9-fluorenyl)methoxycarbonyl]-4-chloro-L-phenylalanine there was obtained 2(RS)-
5 [[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl-L- α -glutamyl]-4-chloro-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 893.3 [M+H].

10

Example 62

In an analogous manner to that described in Example 4, by replacing N-[(9-fluorenyl)methoxycarbonyl]-3-(2-naphthyl)-D-alanine with N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -
15 glutamic acid, by replacing N-[(9-fluorenyl)methoxycarbonyl]-O-tert-butyl-L- α -aspartic acid with N-(benzyloxycarbonyl)-O-tert-butyl-L- α -aspartic acid and by omitting the reaction with tert-butyl succinate there was obtained 2(RS)-[[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-
20 phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde as a white solid; MS: m/e 907.4 [M+H].

Example 63

25 88 mg (0.09 mmol) of N²-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide were dissolved in 5 ml of trifluoroacetic acid and 5 ml of dichloro-
30 methane. 5 drops of water were added and the solution was stirred at room temperature for 4 hours. The solution was diluted with toluene and evaporated. The residue was triturated with diethyl ether and the resulting solid was filtered off and dried to give 72 mg of 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-2-methyl-L-
35 phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-butenylboronic acid; MS: m/e 863 [M+H-H₂O]⁺.

The starting material was prepared as follows:

- i) In an analogous manner to Example 1 iii)-x), by replacing N-[(9-fluorenyl)methoxycarbonyl]-2-methyl-L-phenylalanine with
5 N-[(9-fluorenyl)methoxycarbonyl]-4-chloro-2-methyl-L-phenylalanine there was obtained N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine as a white solid; MS: m/e 952 [M+H]⁺.
- 10 ii) 0.18 g (0.19 mmol) of N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucine was dissolved in 2 ml of dimethylformamide and
15 5 ml of dichloromethane. 0.1 ml (0.94 mmol) of N-methylmorpholine was added and the solution was cooled to -15°C under a nitrogen atmosphere. 35 mg (0.25 mmol) of isobutylchloroformate were added and the solution was stirred for 10 minutes at -10°C. 0.12 g (0.38 mmol) α (RS)-allyl-4,4,5,5-tetramethyl-
20 1,3,2-dioxaborolane-2-methylamine trifluoroacetate (1:1) was added and the mixture was stirred at room temperature for 2 hours. The solution was diluted with dichloromethane, washed with 2M hydrochloric acid and water and dried over anhydrous sodium sulphate. After evaporation there was obtained 0.18 g of
25 N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide in the form of a white solid; MS: m/e 1131.6 [M+H]⁺.
- 30 iii) 166 mg (0.147 mmol) of N²-[N-[N-[N-[N-[3-(tert-butoxycarbonyl)propionyl]-O-tert-butyl-L- α -aspartyl]-O-tert-butyl-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-
35 3-butenyl]-L-leucinamide were dissolved in 5 ml of trifluoroacetic acid and 5 ml of dichloromethane. The solution was stirred at room temperature for 30 minutes, then diluted with toluene and evaporated. The residue was triturated with ether

and the resulting solid was filtered off, dried and then redissolved in 5 ml of trifluoroacetic acid and 5 ml of dichloromethane. The solution was stirred at room temperature for 30 minutes, diluted with toluene and evaporated. The residue
5 was triturated with diethyl ether and the resulting solid was filtered off and dried to give 100 mg of N²-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-N¹-[1(RS)-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-butenyl]-L-leucinamide
10 as a white solid; MS: m/e 863 [M+H-100]⁺.

The following Examples illustrate pharmaceutical preparations containing compounds of formula I:

15

Example A

Tablets containing the following ingredients may be produced in a conventional manner:

| <u>Ingredient</u> | <u>Per tablet</u> |
|-----------------------|-------------------|
| Compound of formula I | 10.0 mg |
| Lactose | 125.0 mg |
| Corn starch | 75.0 mg |
| Talc | 4.0 mg |
| Magnesium stearate | <u>1.0 mg</u> |
| Total weight | <u>215.0 mg</u> |

20

Example B

Capsules containing the following ingredients may be produced in a conventional manner:

5

| <u>Ingredient</u> | <u>Per capsule</u> |
|-----------------------|--------------------|
| Compound of formula I | 10.0 mg |
| Lactose | 165.0 mg |
| Corn starch | 20.0 mg |
| Talc | <u>5.0 mg</u> |
| Capsule fill weight | <u>200.0 mg</u> |

**Figure 1 - Nucleotid sequence of pMAL -NS3''Gly12
NS4A plasmid**

```

      1 CCGACACCAT CGAATGGTGC AAAACCTTTC GCGGTATGGC
5  ATGATAGCGC

      51 CCGGAAGAGA GTCAATTCAG GGTGGTGAAT GTGAAACCAG
   TAACGTTATA

     101 CGATGTCGCA GAGTATGCCG GTGTCTCTTA TCAGACCGTT
10  TCCCGCGTGG

     151 TGAACCAGGC CAGCCACGTT TCTGCGAAAA CGCGGGAAAA
   AGTGGAAGCG
15

     201 GCGATGGCGG AGCTGAATTA CATTCCCAAC CGCGTGGCAC
   AACAACTGGC

     251 GGGCAAACAG TCGTTGCTGA TTGGCGTTGC CACCTCCAGT
20  CTGGCCCTGC

     301 ACGCGCCGTC GCAAATTGTC GCGGCGATTA AATCTCGCGC
   CGATCAACTG

     351 GGTGCCAGCG TGGTGGTGTG GATGGTAGAA CGAAGCGGCG
25  TCGAAGCCTG

     401 TAAAGCGGCG GTGCACAATC TTCTCGCGCA ACGCGTCAGT
   GGGCTGATCA
30

     451 TTAACTATCC GCTGGATGAC CAGGATGCCA TTGCTGTGGA
   AGCTGCCTGC

     501 ACTAATGTTC CGGCGTTATT TCTTGATGTC TCTGACCAGA
35  CACCCATCAA

     551 CAGTATTATT TTCTCCCATG AAGACGGTAC GCGACTGGGC
   GTGGAGCATC

     601 TGGTCGCATT GGGTCACCAG CAAATCGCGC TGTTAGCGGG
40  CCCATTAAGT

     651 TCTGTCTCGG CGCGTCTGCG TCTGGCTGGC TGGCATAAAT
   ATCTCACTCG
45

     701 CAATCAAATT CAGCCGATAG CGGAACGGGA AGGCGACTGG
   AGTGCCATGT

     751 CCGGTTTTCA ACAAACCATG CAAATGCTGA ATGAGGGCAT
50  CGTTCCCACT

     801 GCGATGCTGG TTGCCAACGA TCAGATGGCG CTGGGCGCAA
   TGC GCGCCAT

     851 TACCGAGTCC GGGCTGCGCG TTGGTGCGGA TATCTCGGTA
55  GTGGGATACG
```

901 ACGATACCGA AGACAGCTCA TGTTATATCC CGCCGTTAAC
CACCATCAAA

5 951 CAGGATTTTC GCCTGCTGGG GCAAACCAGC GTGGACCGCT
TGCTGCAACT

1001 CTCTCAGGGC CAGGCGGTGA AGGGCAATCA GCTGTTGCCC
GTCTCACTGG

10 1051 TGAAAAGAAA AACCACCCTG GCGCCCAATA CGCAAACCGC
CTCTCCCCGC

1101 GCGTTGGCCG ATTCATTAAT GCAGCTGGCA CGACAGGTTT
CCCGACTGGA

15 1151 AAGCGGGCAG TGAGCGCAAC GCAATTAATG TGAGTTAGCT
CACTCATTAG

1201 GCACAATTCT CATGTTTGAC AGCTTATCAT CGACTGCACG
20 GTGCACCAAT

1251 GCTTCTGGCG TCAGGCAGCC ATCGGAAGCT GTGGTATGGC
TGTGCAGGTC

25 1301 GTAAATCACT GCATAATTCG TGTCGCTCAA GGCGCACTCC
CGTTCTGGAT

1351 AATGTTTTTT GCGCCGACAT CATAACGGTT CTGGCAAATA
TTCTGAAATG

30 1401 AGCTGTTGAC AATTAATCAT CGGCTCGTAT AATGTGTGGA
ATTGTGAGCG

1451 GATAACAATT TCACACAGGA AACAGCCAGT CCGTTTAGGT
35 GTTTTCACGA

1501 GCACTTCACC AACAAGGACC ATAGATTATG AAAACTGAAG
AAGGTAAACT

40 1551 GGTAATCTGG ATTAACGGCG ATAAAGGCTA TAACGGTCTC
GCTGAAGTCG

1601 GTAAGAAATT CGAGAAAGAT ACCGGAATTA AAGTCACCGT
TGAGCATCCG

45 1651 GATAAACTGG AAGAGAAATT CCCACAGGTT GCGGCAACTG
GCGATGGCCC

1701 TGACATTATC TTCTGGGCAC ACGACCGCTT TGGTGGCTAC
50 GCTCAATCTG

1751 GCCTGTTGGC TGAAATCACC CCGGACAAAG CGTTCCAGGA
CAAGCTGTAT

55 1801 CCGTTTACCT GGGATGCCGT ACGTTACAAC GGCAAGCTGA
TTGCTTACCC

Start MBP

1851 GATCGCTGTT GAAGCGTTAT CGCTGATTTA TAACAAAGAT
CTGCTGCCGA

5 1901 ACCCGCCAAA AACCTGGGAA GAGATCCCGG CGCTGGATAA
AGAACTGAAA

1951 GCGAAAGGTA AGAGCGCGCT GATGTTCAAC CTGCAAGAAC
CGTACTTCAC

10 2001 CTGGCCGCTG ATTGCTGCTG ACGGGGGTTA TGC GTTCAAG
TATGAAAACG

2051 GCAAGTACGA CATTAAAGAC GTGGGCGTGG ATAACGCTGG
CGCGAAAGCG

15 2101 GGTCTGACCT TCCTGGTTGA CCTGATTAAA AACAAACACA
TGAATGCAGA

2151 CACCGATTAC TCCATCGCAG AAGCTGCCTT TAATAAAGGC
20 GAAACAGCGA

2201 TGACCATCAA CGGCCCCGTGG GCATGGTCCA ACATCGACAC
CAGCAAAGTG

25 2251 AATTATGGTG TAACGGTACT GCCGACCTTC AAGGGTCAAC
CATCCAAACC

2301 GTTCGTTGGC GTGCTGAGCG CAGGTATTAA CGCCGCCAGT
CCGAACAAAG

30 2351 AGCTGGCAAA AGAGTTCCTC GAAAACTATC TGCTGACTGA
TGAAGGTCTG

2401 GAAGCGGTTA ATAAAGACAA ACCGCTGGGT GCCGTAGCGC
35 TGAAGTCTTA

2451 CGAGGAAGAG TTGGCGAAAG ATCCACGTAT TGCCGCCACC
ATGGAAAACG

40 2501 CCCAGAAAGG TGAAATCATG CCGAACATCC CGCAGATGTC
CGCTTTCTGG

2551 TATGCCGTGC GTACTGCGGT GATCAACGCC GCCAGCGGTC
GTCAGACTGT

45 2601 CGATGAAGCC CTGAAAGACG CGCAGACTAA TTCGAGCTCG
AACAACAACA

2651 ACAATAACAA TAACAACAAC CTCGGGATCG AGGGAAGGAT
50 TTCAGAATTC

EcoRI

2701 ATGGGGAGGG AGATACATCT GGGACCGGCA GACAGCCTTG
AAGGGCAGGG

55 **NS2/3 (**

2751 GTGGCGACTC CTCGCGCATA TTACGGCCTA CTCTCAACAG
ACGCGGGGCC

2801 TACTTGGCTG CATCATCACT AGCCTCACAG GCCGGGACAG
GAACCAGGTC

2851 GAGGGGGAGG TCCAAATGGT CTCCACCGCA ACACAATCTT
5 TCCTGGCGAC

2901 CTGCGTCAAT GGCCTGTGTT GGACTGTCTA TCATGGTGCC
GGCTCAAAGA

2951 CCCTTGCCGG CCCAAAGGGC CCAATCACCC AAATGTACAC
10 CAATGTGGAC

3001 CAGGACCTCG TCGGCTGGCA AGCGCCCCC GGGGCGCGCT
CCTTGACACC

3051 ATGCACCTGC GGCAGCTCAG ACCTTTACTT GGTCACGAGG
CATGCCGATG

3101 TCATTCCGGT GCGCCGGCGG GGCACAGCA GGGGAAGCCT
20 ACTCTCCCCC

3151 AGGCCCGTCT CCTACTTGAA GGGCTCTTCG GGCAGTCCAC
TGCTCTGCCC

3201 CTCGGGGCAC GCTGTGGGCA TCTTCCGGGC TGCCGTGTGC
25 ACCCGAGGGG

3251 TTGCGAAGGC GGTGGACTTT GTACCCGTCG AGTCTATGGA
AACCACTATG

3301 CGGTCCCCGG TCTTCACGGA CAACTCGTCC CCTCCGGCCG
TATGCATGGG

Eag I

linker (

3351 AGGAGGAGGA GGAGGAGGAG GAGGAGGAGG AGGATCCATG
35 AGCACCTGGG

BamHI

NS4A (

3401 TGCTAGTAGG CGGAGTCCTA GCAGCTCTGG CCGCGTATTG
40 CCTGACAACA

3451 GGCAGCGTGG TCATTGTGGG CAGGATCGTC TTGTCCGGAA
AGCCGGCCAT

3501 CATTCCCGAC AGGGAAGTCC TCTACCGGGA GTTCGATGAG
45 ATGGAAGAGT

3551 GCTAGAAGCT TGGCACTGGC CGTCGTTTTA CAACGTCGTG
ACTGGGAAAA

End HindIII

3601 CCCTGGCGTT ACCCAACTTA ATCGCCTTGC AGCACATCCC
50 CCTTTCGCCA

3651 GCTGGCGTAA TAGCGAAGAG GCCCGCACCG ATCGCCCTTC
55 CCAACAGTTG

3701 CGCAGCCTGA ATGGCGAATG GCAGCTTGGC TGTTTTGGCG
GATGAGATAA

5 3751 GATTTTCAGC CTGATACAGA TTAAATCAGA ACGCAGAAGC
GGTCTGATAA

3801 AACAGAATTT GCCTGGCGGC AGTAGCGCGG TGGTCCCACC
TGACCCCATG

10 3851 CCGAACTCAG AAGTGAAACG CCGTAGCGCC GATGGTAGTG
TGGGGTCTCC

3901 CCATGCGAGA GTAGGGAAC TCCAGGCATC AAATAAAACG
AAAGGCTCAG

15 3951 TCGAAAGACT GGGCCTTTCG TTTTATCTGT TGTTTGTCGG
TGAACGCTCT

4001 CCTGAGTAGG ACAAATCCGC CGGGAGCGGA TTTGAACGTT
20 GCGAAGCAAC

4051 GGCCCGGAGG GTGGCGGGCA GGACGCCCGC CATAAACTGC
CAGGCATCAA

25 4101 ATTAAGCAGA AGGCCATCCT GACGGATGGC CTTTTTGCGT
TTCTACAAAC

4151 TCTTTTTGTT TATTTTTCTA AATACATTCA AATATGTATC
CGCTCATGAG

30 4201 ACAATAACCC TGATAAATGC TTCAATAATA TTGAAAAAGG
AAGAGTATGA

4251 GTATTCAACA TTTCCGTGTC GCCCTTATTC CCTTTTTTGC
35 GGCATTTTGC

4301 CTTCCTGTTT TTGCTCACCC AGAAACGCTG GTGAAAGTAA
AAGATGCTGA

40 4351 AGATCAGTTG GGTGCACGAG TGGGTTACAT CGAACTGGAT
CTCAACAGCG

4401 GTAAGATCCT TGAGAGTTTT CGCCCCGAAG AACGTTCTCC
AATGATGAGC

45 4451 ACTTTTAAAG TTCTGCTATG TGGCGCGGTA TTATCCCGTG
TTGACGCCGG

4501 GCAAGAGCAA CTCGGTCGCC GCATACACTA TTCTCAGAAT
50 GACTTG GTTG

4551 AGTACTCACC AGTCACAGAA AAGCATCTTA CGGATGGCAT
GACAGTAAGA

55 4601 GAATTATGCA GTGCTGCCAT AACCATGAGT GATAAACTG
CGGCCAACTT

4651 ACTTCTGACA ACGATCGGAG GACCGAAGGA GCTAACCGCT
TTTTTGCACA

5 4701 ACATGGGGGA TCATGTAACT CGCCTTGATC GTTGGGAACC
GGAGCTGAAT

4751 GAAGCCATAC CAAACGACGA GCGTGACACC ACGATGCCTG
TAGCAATGGC

10 4801 AACAACTTG CGCAAATAT TAACTGGCGA ACTACTTACT
CTAGCTTCCC

4851 GGCAACAATT AATAGACTGG ATGGAGGCGG ATAAAGTTGC
AGGACCACTT

15 4901 CTGCGCTCGG CCCTTCCGGC TGGCTGGTTT ATTGCTGATA
AATCTGGAGC

4951 CCGTGAGCGT GGGTCTCGCG GTATCATTGC AGCACTGGGG
20 CCAGATGGTA

5001 AGCCCTCCCG TATCGTAGTT ATCTACACGA CGGGGAGTCA
GGCAACTATG

25 5051 GATGAACGAA ATAGACAGAT CGCTGAGATA GGTGCCTCAC
TGATTAAGCA

5101 TTGGTAACTG TCAGACCAAG TTTACTCATA TATACTTTAG
ATTGATTTAC

30 5151 CCCGGTTGAT AATCAGAAAA GCCCCAAAAA CAGGAAGATT
GTATAAGCAA

5201 ATATTTAAAT TGTAACGTT AATATTTTGT TAAAATTTCG
35 GTTAAATTTT

5251 TGTTAAATCA GCTCATTTTT TAACCAATAG GCCGAAATCG
GCAAAATCCC

40 5301 TTATAAATCA AAAGAATAGC CCGAGATAGG GTTGAGTGTT
GTTCCAGTTT

5351 GGAACAAGAG TCCACTATTA AAGAACGTGG ACTCCAACGT
CAAAGGGCGA

45 5401 AAAACCGTCT ATCAGGGCGA TGGCCCACTA CGTGAACCAT
CACCCAAATC

5451 AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC ACTAAATCGG
50 AACCTAAAG

5501 GGAGCCCCCG ATTTAGAGCT TGACGGGGAA AGCCGGCGAA
CGTGGCGAGA

55 5551 AAGGAAGGGA AGAAAGCGAA AGGAGCGGGC GCTAGGGCGC
TGGCAAGTGT

5601 AGCGGTCACG CTGCGCGTAA CCACCACACC CGCCGCGCTT
AATGCGCCGC

5 5651 TACAGGGCGC GTAAAAGGAT CTAGGTGAAG ATCCTTTTGT
ATAATCTCAT

5701 GACCAAAATC CCTTAACGTG AGTTTTTCGT CCACTGAGCG
TCAGACCCCG

10 5751 TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
GCGCGTAATC

5801 TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG
TTTGTTTGCC

15 5851 GGATCAAGAG CTACCAACTC TTTTTCGAA GGTAAGTGGC
TTCAGCAGAG

5901 CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT
20 AGGCCACCAC

5951 TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
TAATCCTGTT

25 6001 ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC
GGGTTGGACT

6051 CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
AACGGGGGGT

30 6101 TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG
AACTGAGATA

6151 CCTACAGCGT GAGCTATGAG AAAGCGCCAC GCTTCCCGAA
35 GGGAGAAAGG

6201 CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA
GCGCACGAGG

40 6251 GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
TCGGGTTTCG

6301 CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA
GGGGGGCGGA

45 6351 GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
CCTGGCCTTT

6401 TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC
50 CTGATTCTGT

6451 GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATAACCGT
CGCCGCAGCC

55 6501 GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA
AGAGCGCCTG

6551 ATGCGGTATT TTCTCCTTAC GCATCTGTGC GGTATTTTAC
ACCGCATATG

5 6601 GTGCACTCTC AGTACAATCT GCTCTGATGC CGCATAGTTA
AGCCAGTATA

6651 CACTCCGCTA TCGCTACGTG ACTGGGTCAT GGCTGCGCCC
CGACACCCGC

10 6701 CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC
GGCATCCGCT

6751 TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC
AGAGGTTTTTC

15 6801 ACCGTCATCA CCGAAACGCG CGAGGCAGCT GCGGTAAAGC
TCATCAGCGT

6851 GGTCGTGCAG CGATTACAG ATGTCTGCCT GTTCATCCGC
20 GTCCAGCTCG

6901 TTGAGTTTCT CCAGAAGCGT TAATGTCTGG CTTCTGATAA
AGCGGGCCAT

25 6951 GTTAAGGGCG GTTTTTTCCT GTTTGGTCAC TTGATGCCTC
CGTGTAAGGG

7001 GGAATTTCTG TTCATGGGGG TAATGATACC GATGAAACGA
GAGAGGATGC

30 7051 TCACGATACG GGTTACTGAT GATGAACATG CCCGGTTACT
GGAACGTTGT

7101 GAGGGTAAAC AACTGGCGGT ATGGATGCGG CGGGACCAGA
35 GAAAAATCAC

7151 TCAGGGTCAA TGCCAGCGCT TCGTTAATAC AGATGTAGGT
GTTCCACAGG

40 7201 GTAGCCAGCA GCATCCTGCG ATGCAGATCC GGAACATAAT
GGTGCAGGGC

7251 GCTGACTTCC GCGTTTCCAG ACTTTACGAA ACACGGAAAC
CGAAGACCAT

45 7301 TCATGTTGTT GCTCAGGTCG CAGACGTTTT GCAGCAGCAG
TCGCTTCACG

7351 TTCGCTCGCG TATCGGTGAT TCATTCTGCT AACCAGTAAG
50 GCAACCCCGC

7401 CAGCCTAGCC GGGTCCTCAA CGACAGGAGC ACGATCATGC
GCACCCGTGG

55 7451 CCAGGACCCA ACGCTGCCCG AAATT

Figur 2 - Amino acid sequence of MBP-NS3''-gly₁₂-4A enzyme

5

1 MKTEEGKLVI WINGDKGYNG LAEVGKKFEK DTGIKVTVEH
PDKLEEKFPQ

10 51 VAATGDGPD IFWAHDRFGG YAQSGLLAEI TPDKAFQDKL
YPFTWDAVRY

15 101 NGKLIAYPIA VEALSIIYNK DLLPNPPKTW EEIPALDKEL
KAKGKSALMF

151 NLQEPYFTWP LIAADGGYAF KYENGKYDIK DVGVDNAGAK
AGLTFLVDLI

20 201 KKNKHMNADTD YSIAEAAFNK GETAMTINGP WAWSNIDTSK
VNYGVTVLPT

25 251 FKGQPSKPFV GVLSAGINAA SPNKELAKEF LENYLLTDEG
LEAVNKDKPL

30 301 GAVALKSIEE ELAKDPRIAA TMENAQKGEI MPNIPQMSAF
WYAVRTAVIN

351 AASGRQTVDE ALKDAQTNSS SNNNNNNNNNN NLGIEGRISE
FMGREIHLGP

30 **NS2/3 (**
401 ADSLEGQGWR LLAHITAYSQ QTRGLLGCI I TSLTGRDRNQ
VEGEVQMVST

35 451 ATQSFLATCV NGVCWTVYHG AGSKTLAGPK GPITQMYTNV
DQDLVGWQAP

501 PGARSLTPCT CGSSDLYLVT RHADVIVRR RGDSRGSLLS
PRPVSYLKGS

40 551 SGGPLLCPSG HAVGIFRAAV CTRGVAKAVD FVPVESMETT
MRSPVFTDNS

45 601 SPPAVCMGGG GGGGGGGGGS MSTWVLVGGV LAALAAAYCLT
TGSVVIVGRI

Linker (NS4A (
651 VLSGKPAIIP DREVLYREFD EMEEC

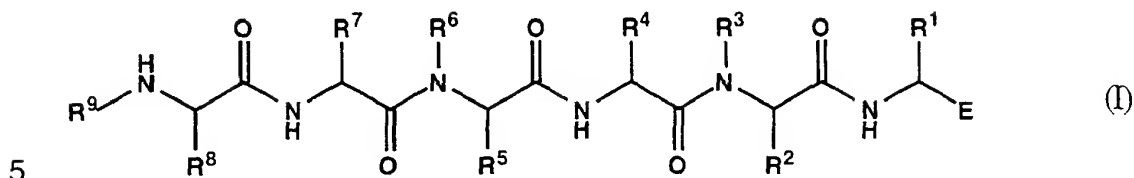
50 Amino acids 1-391 - Maltose binding protein and other
sequences derived from New England Biolabs vector pMALTM-c2

Amino acids 393-605 and 622-675 - HCV-derived sequences
(amino acids 1007-1219 and 1658-1711 of HCV polyprotein
respectively)

55 Amino acids 606-621 - linker region

Claims

1. Compounds of the general formula



wherein

E represents CHO or B(OH)₂;

10 R¹ represents lower alkyl, halo-lower alkyl, cyano-lower alkyl, lower alkylthio-lower alkyl, aryl-lower alkylthio-lower alkyl, aryl-lower alkyl, heteroaryl-lower alkyl, lower alkenyl or lower alkynyl;

15 R² represents lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, aminocarbonyl-lower alkyl or lower cycloalkyl-lower alkyl; and

R³ represents hydrogen or lower alkyl; or

R² and R³ together represent di- or trimethylene optionally substituted by hydroxy;

20 R⁴ represents lower alkyl, hydroxy-lower alkyl, lower cycloalkyl-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, lower alkylthio-lower alkyl, cyano-lower alkylthio-lower alkyl, aryl-lower alkylthio-lower alkyl, lower alkenyl, aryl or lower cycloalkyl;

25 R⁵ represents lower alkyl, hydroxy-lower alkyl, lower alkylthio-lower alkyl, aryl-lower alkyl, aryl-lower alkylthio-lower alkyl, cyano-lower alkylthio-lower alkyl or lower cycloalkyl;

R⁶ represents hydrogen or lower alkyl;

30 R⁷ represent lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl, aryl-lower alkyl, lower cycloalkyl-lower alkyl or lower cycloalkyl;

R⁸ represents lower alkyl, hydroxy-lower alkyl, carboxy-lower alkyl or aryl-lower alkyl; and

35 R⁹ represents lower alkylcarbonyl, carboxy-lower alkylcarbonyl, arylcarbonyl, lower alkylsulphonyl, arylsulphonyl, lower alkoxy-carbonyl or aryl-lower

alkoxycarbonyl,
and salts of acidic compounds of formula I with bases.

2. Compounds of the general formula I according to
5 claim 1.

3. Compounds according to claim 1, wherein R¹
represents lower alkyl, halo-lower alkyl, lower alkylthio-lower
alkyl, aryl-lower alkylthio-lower alkyl, heteroaryl-lower alkyl,
10 lower alkenyl or lower alkynyl.

4. Compounds according to claim 3, wherein the halo-
lower alkyl group is fluoro-lower alkyl.

15 5. Compounds according to claim 3, wherein the hetero-
aryl-lower alkyl group is thienyl-lower alkyl or furyl-lower
alkyl.

6. Compounds according to any one of claims 1 to 5,
20 wherein R² represents lower alkyl, lower cycloalkyl-lower alkyl
or aryl-lower alkyl.

7. Compounds according to any one of claims 1 to 6,
wherein R³ represents hydrogen.

25

8. Compounds according to any one of claims 1 to 5,
wherein R² and R³ together represent trimethylene optionally
substituted by hydroxy.

30 9. Compounds according to any one of claims 1 to 8,
wherein R⁴ represents lower alkyl, lower cycloalkyl-lower alkyl,
aryl-lower alkyl, aryl or lower cycloalkyl.

10. Compounds according to any one of claims 1 to 9,
35 wherein R⁵ represents aryl-lower alkyl or lower cycloalkyl.

11. Compounds according to any one of claims 1 to 10,
wherein R⁶ represents hydrogen.

12. Compounds according to any one of claims 1 to 11, wherein R⁷ represents lower alkyl, carboxy-lower alkyl, aryl-lower alkyl or hydroxy-lower alkyl.

5

13. Compounds according to any one of claims 1 to 12, wherein R⁸ represents hydroxy-lower alkyl, carboxy-lower alkyl or aryl-lower alkyl.

10

14. Compounds according to any one of claims 1 to 13, wherein R⁹ represents lower alkylcarbonyl or carboxy-lower alkylcarbonyl.

15. A compound according to claim 1 selected from:

15

2(S)-[[N-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butyraldehyde;

2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4-difluorovaleraldehyde;

2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;

2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(methylthio)propionaldehyde;

2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(butylthio)propionaldehyde;

2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentenaldehyde;

2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-pentynal;

2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-

- leucyl]amino]-4-hexynal;
 3-(benzylthio)-2(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propionaldehyde;
 5 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(2-thienyl)propionaldehyde;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-(3-thienyl)propionaldehyde; and
 10 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-3-(2-naphthyl)-D-alanyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde.
- 15 16. A compound according to claim 1, selected from:
- 2(RS)-[[N-[N-[N-[N-(3-Carboxypropionyl)-L-seryl-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 20 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]hexanal;
 (Z)-2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4-hexenal;
 25 2(RS)-[[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 30 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 35 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-methylhexanal;
 2(S)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L-

- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-5-hexenal;
 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-norleucyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 5 2(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-D-2-cyclohexylglycyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde; and
 2(RS)-[[N-[N-[N-[N-(4-acetamidobenzoyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-4,4,4-trifluorobutyraldehyde;
 10

17. A compound according to claim 1, selected from:

- 15 1(RS)-[[N-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]propylboronic acid;
 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]butylboronic acid; and
 20 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-butenylboronic acid.

25 18. A compound according to claim 1, selected from:

- 1(RS)-[[N-[N-[N-[N-(3-Carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-4-chloro-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]-3-butenylboronic acid;
 30 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-3-cyclopentyl-L-alanyl]amino]-3-butenylboronic acid;
 1(R)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl]-L-leucyl]amino]pentylboronic acid;
 35 1(RS)-[[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-L- α -glutamyl]-2-methyl-L-phenylalanyl]-L-2-cyclohexylglycyl]-L-leucyl]amino]propylboronic acid;

1 (RS)-[[N-[N-[N-[N-[N-(3-carboxypropionyl)-L- α -aspartyl]-
L- α -glutamyl]-L-2-cyclohexylglycyl]-3-methyl-L-valyl]-L-
leucyl]amino]propylboronic acid; and

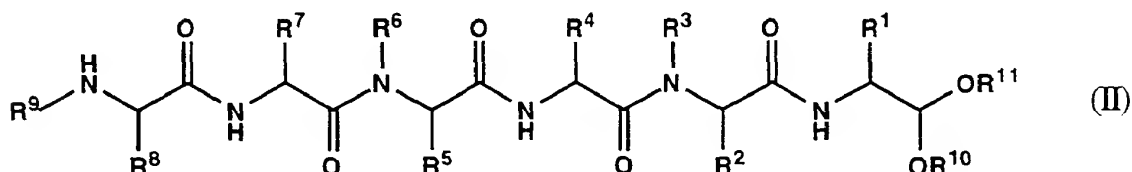
1 (RS)-[[N-[N-[N-[N-[N-(benzyloxycarbonyl)-L- α -aspartyl]-D-
5 valyl]-2-methyl-L-phenylalanyl]-3-methyl-L-valyl-L-leucyl]-
amino]propylboronic acid.

19. A compound according to any one of claims 1 to 18 for
use as a therapeutically active substance, especially as an
10 antiviral agent and particularly as an agent against Hepatitis C,
Hepatitis G or human GB viruses.

20. A process for the manufacture of a compound
according to any one of claims 1 to 18 and of salts of those
15 compounds which are acidic with bases which process comprises

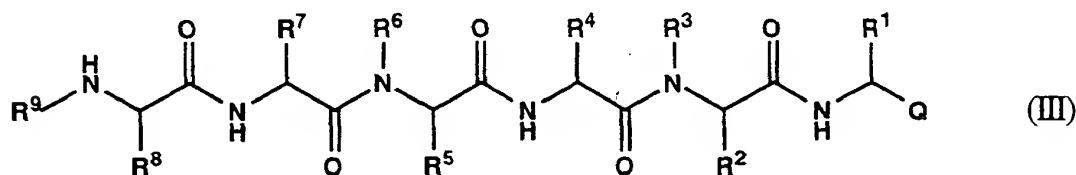
a) for the manufacture of a compound of formula I in which E
represents CHO, deacetalizing and, where required, deprotecting
an acetal of the general formula

20



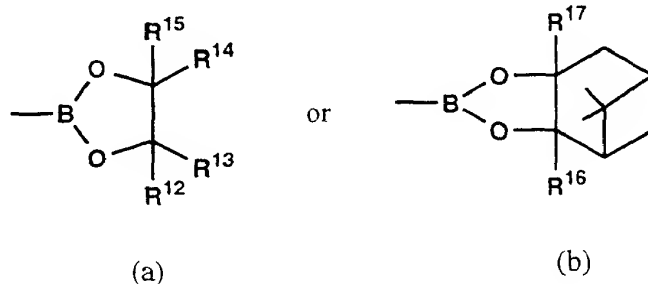
wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ have the
significance given in claim 1, provided that any carboxy,
25 hydroxy and/or aminocarbonyl group(s) present is/are in
protected form, and R¹⁰ and R¹¹ each represent lower alkyl,

b) for the manufacture of a compound of formula I in which E
represents B(OH)₂, ring opening and, where required, deprotecting
30 a substituted dioxaborolane of the general formula



wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ and R⁹ have the significance given in claim 1, provided that any carboxy, hydroxy and/or aminocarbonyl group(s) present may be in protected form, and Q represents a group of the formula

5



wherein R¹², R¹³, R¹⁴ and R¹⁵ each represent hydrogen or lower alkyl and R¹⁶ and R¹⁷ each represent hydrogen or lower alkyl.

10

and

c) if desired, converting an acidic compound of formula I obtained into a salt with a base.

15

21. A process according to claim 20, wherein the acetal of formula II or substituted dioxaborolane of formula III in which Q represents a group of formula (a) is bonded to a solid phase peptide synthesis resin.

20

22. Acetals of formula II given in claim 20.

23. Substituted dioxaborolanes of formula III given in claim 20.

25

24. A medicament, especially an antiviral medicament, particularly a medicament against Hepatitis C, Hepatitis G or human GB viruses, containing a compound according to any one of claims 1 to 18 in association with a compatible pharmaceutical carrier.

30

25. The use of a compound according to any one of claims 1 to 18 for the production of an antiviral medicament, especially a medicament against Hepatitis C, Hepatitis G or human GB viruses.

5

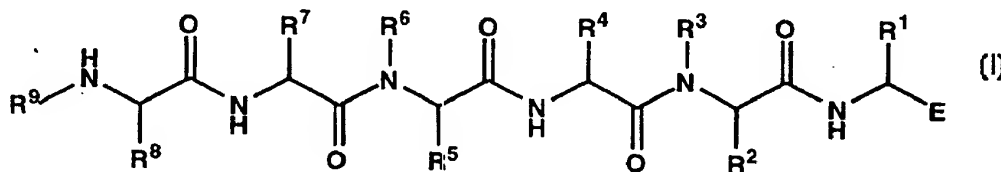
26. The invention as hereinbefore described.

* * *

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| (21) International Application Number: PCT/EP97/06189 (22) International Filing Date: 7 November 1997 (07.11.97) (30) Priority Data: 9623908.2 18 November 1996 (18.11.96) GB (71) Applicant: F. HOFFMANN-LA ROCHE AG [CH/CH]; Grenzacherstrasse 124, CH-4070 Basle (CH). (72) Inventors: ATTWOOD, Michael, Richard; 22 Benslow Rise, Hitchin, Herts. SG4 9QX (GB). HURST, David, Nigel; 23 Cubitts Close, Welwyn, Herts. AL6 0DL (GB). JONES, Philip, Stephen; 58 Digswell Rise, Welwyn Garden City, Herts. AL8 7PW (GB). KAY, Paul, Brittain; 6 Mercia Road, Baldock, Herts. SG7 6RZ (GB). RAYNHAM, Tony, Michael; Braemar, High Road, Laindon, Basildon, Essex SS16 6BU (GB). WILSON, Francis, Xavier; 11 Great Conduit, Welwyn Garden City, Herts. AL7 2DH (GB). (74) Agent: MEZGER, Wolfgang; Grenzacherstrasse 124, CH-4070 Basle (CH). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 16 July 1998 (16.07.98) | |

(54) Title: ANTIVIRAL PEPTIDE DERIVATIVES



(57) Abstract

The invention provides amino acid derivatives of formula (I) wherein E represents CHO or B(OH)₂; R¹ represents lower alkyl (optionally substituted by halo, cyano, lower alkylthio, aryl-lower alkylthio, aryl or heteroaryl), lower alkenyl or lower alkynyl; R² represents lower alkyl optionally substituted by hydroxy, carboxy, aryl, aminocarbonyl or lower cycloalkyl; and R³ represents hydrogen or lower alkyl; or R² and R³ together represent di- or trimethylene optionally substituted by hydroxy; R⁴ represents lower alkyl (optionally substituted by hydroxy, lower cycloalkyl, carboxy, aryl, lower alkylthio, cyano-lower alkylthio or aryl-lower alkylthio), lower alkenyl, aryl or lower cycloalkyl; R⁵ represents lower alkyl (optionally substituted by hydroxy, lower alkylthio, aryl, aryl-lower alkylthio or cyano-lower alkylthio) or lower cycloalkyl; R⁶ represents hydrogen or lower alkyl; R⁷ represents lower alkyl (optionally substituted by hydroxy, carboxy, aryl or lower cycloalkyl) or lower cycloalkyl; R⁸ represents lower alkyl optionally substituted by hydroxy, carboxy or aryl; and R⁹ represents lower alkylcarbonyl, carboxy-lower alkylcarbonyl, arylcarbonyl, lower alkylsulphonyl, arylsulphonyl, lower alkoxy carbonyl or aryl-lower alkoxy carbonyl, and salts of acidic compounds of formula (I) with bases, which are viral proteinase inhibitors useful as antiviral agents, especially for the treatment or prophylaxis of infections caused by Hepatitis C, Hepatitis G and human GB viruses.

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 97/06189

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K7/06 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| A | STEINKÜHLER E.A.: "Activity of purified Hepatitis C virus protease NS3 on peptide substrates" JOURNAL OF VIROLOGY., vol. 70, no. 10, October 1996, ICAN SOCIETY FOR MICROBIOLOGY US, pages 6694-6700, XP002064087 see the whole document ---- | 1-26 |
| A | WO 92 22570 A (CHIRON CORP) 23 December 1992 see the whole document ---- | 1-26 |
| A | WO 95 15766 A (HOUGHTEN PHARM INC) 15 June 1995 see the whole document ---- | 1-26 |
| -/-- | | |



Further documents are listed in the continuation of box C.



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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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|----------|--|-----------------------|
| P, A | <p>WO 97 08304 A (ANGELETTI P IST RICHERCHE BIO :STEINKUEHLER CHRISTIAN (IT); PESSI) 6 March 1997 see the whole document -----</p> | 1-26 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 97/06189

| Patent document cited in search report | | Publication date | Patent family member(s) | | Publication date |
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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|---|-----------|---|
| (51) International Patent Classification ⁶ : A61K 31/44, 31/495 | A1 | (11) International Publication Number: WO 97/36587 (43) International Publication Date: 9 October 1997 (09.10.97) |
| (21) International Application Number: PCT/US97/05328 (22) International Filing Date: 31 March 1997 (31.03.97) (30) Priority Data: 60/014,773 3 April 1996 (03.04.96) US 9613599.1 28 June 1996 (28.06.96) GB (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): HEIMBROOK, David, C. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). OLIFF, Allen, I. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). STIRDIVANT, Steven, M. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). | | (81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: A METHOD OF TREATING CANCER (57) Abstract The present invention relates to a method of treating cancer which comprises administering to a mammalian patient a compound which inhibits Raf and a compound which inhibits farnesyl protein transferase. | | |

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TITLE OF THE INVENTION
A METHOD OF TREATING CANCER

BACKGROUND OF THE INVENTION

5 The present invention relates to a method of treating cancer using a combination of a compound which has Raf antagonist activity and a compound which has farnesyl transferase inhibiting activity.

 The Raf antagonist compounds used in the present invention demonstrate anti-cancer activity through antagonism of the kinase, Raf .
10 The *raf* genes code for a family of proteins which can be oncogenically activated through N-terminal fusion, truncation or point mutations. Raf is a member of the MAP Kinase cascade, which also includes MEK's and MAP Kinase (ERK). Raf can be activated and undergoes rapid phosphorylation in response to treatment of cells with PDGF, EGF,
15 insulin, thrombin, endothelin, acidic FGF, CSF1 or TPA, as well as in response to oncoproteins v-fms, v-src, v-sis, Hras and polyoma middle T antigen. Antisense constructs which reduce cellular levels of c-Raf, and hence Raf activity, inhibit the growth of oncogene-transformed rodent fibroblasts in soft agar, while exhibiting little or no general
20 cytotoxicity. Since inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals, these studies suggest that the antagonism of Raf is an effective means by which to treat cancers in which Raf plays a role.

 Examples of cancers where Raf is implicated through
25 overexpression include cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung. More particularly, such examples include histiocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Additional examples include cancers in which overexpression or activation of Raf-activating oncogenes (e.g., *K-ras*,
30 *erb-B*) is observed. More particularly, such cancers include pancreatic and breast carcinoma.

 The Ras protein is part of a signalling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action

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indicate that Ras functions like a G-regulatory protein. In the inactive state, Ras is bound to GDP. Upon growth factor receptor activation, Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, *Ann. Rev. Biochem.* 62:851-891 (1993)). Activation of Ras leads to activation of multiple intracellular signal transduction pathways, including the MAP Kinase pathway and the Rho/Rac pathway (Joneson *et al.*, *Science* 271:810-812).

Mutated *ras* genes are found in many human cancers, including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

The Ras protein is one of several proteins that are known to undergo post-translational modification. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss *et al.*, *Cell*, 62:81-88 (1990); Schaber *et al.*, *J. Biol. Chem.*, 265:14701-14704 (1990); Schafer *et al.*, *Science*, 249:1133-1139 (1990); Manne *et al.*, *Proc. Natl. Acad. Sci USA*, 87:7541-7545 (1990)).

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa¹-Aaa²-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen *et al.*, *Nature* 310:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase, which catalyze the alkylation of the cysteine residue of the

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CAAX motif with a C₁₅ or C₂₀ isoprenoid, respectively. (S. Clarke., *Ann. Rev. Biochem.* 61:355-386 (1992); W.R. Schafer and J. Rine, *Ann. Rev. Genetics* 30:209-237 (1992)). However, direct inhibition of farnesyl-protein transferase would be more specific and attended by
5 fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Other farnesylated proteins include the Ras-related GTP-binding proteins such as Rho, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., *J. Biol. Chem.* 269,
10 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated. James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibitors of farnesyl-protein transferase (FPTase)
15 have been described in two general classes. The first class includes analogs of farnesyl diphosphate (FPP), while the second is related to protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for
20 protein prenylation. (Schaber *et al.*, *ibid*; Reiss *et al.*, *ibid*; Reiss *et al.*, *PNAS*, 88:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl *et al.*, *Science*,
25 260:1934-1937 (1993); Graham, et al., *J. Med. Chem.*, 37, 725 (1994)).

Inhibition of farnesyl-protein transferase has been shown to block the growth of *ras*-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase
30 selectively block the processing of the Ras oncoprotein intracellularly (N.E. Kohl *et al.*, *Science*, 260:1934-1937 (1993) and G.L. James *et al.*, *Science*, 260:1937-1942 (1993). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of *ras*-dependent tumors in nude mice (N.E. Kohl *et al.*, *Proc. Natl. Acad. Sci*

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U.S.A., 91:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in *ras* transgenic mice (N.E. Kohl *et al.*, *Nature Medicine*, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase *in vivo* has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock *et al.*, *ibid*; Casey *et al.*, *ibid*; Schafer *et al.*, *Science* 245:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of polyisoprenoids including farnesyl pyrophosphate. Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells.

A Raf antagonist compound and a farnesyl protein transferase inhibitor are used in the present invention to treat cancer, such as in tumor cells which are not particularly Raf or FPTase dependent. The Raf antagonist compound and a farnesyl protein transferase inhibiting compound are used in combination.

SUMMARY OF THE INVENTION

A method of treating cancer is disclosed which is comprised of administering to a mammalian patient in need of such treatment an effective amount of a Raf antagonist compound and an effective amount of a farnesyl protein transferase inhibiting compound.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treating cancer which is comprised of administering to a mammalian patient in need of such treatment an effective amount of a Raf antagonist compound and an effective amount of a farnesyl protein transferase inhibiting compound. Any compound which antagonizes Raf and any compound which inhibits farnesyl protein transferase can be used.

As used herein the term Raf antagonist is used in the general sense to relate to compounds which antagonize, inhibit or counteract the activity of the *raf* gene or the protein produced in response thereto.

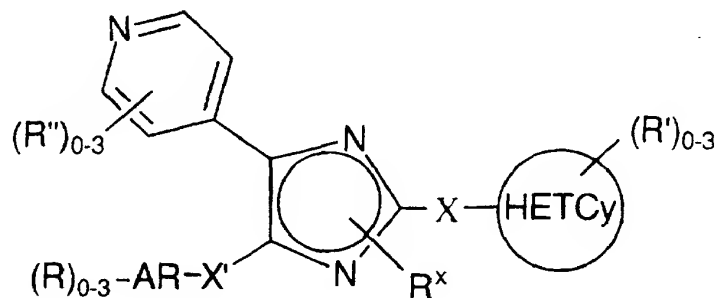
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The term farnesyl protein transferase inhibiting compound is likewise used in the general sense and refers to compounds which antagonize, inhibit or counteract the activity of the gene coding farnesyl protein transferase or the protein produced in response thereto.

5 Cancers which are treatable in accordance with the invention described herein include cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx, liver and lung. More particularly, such cancers include histiocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Additional examples include cancers in which
 10 overexpression or activation of Raf-activating oncogenes (e.g., *K-ras*, *erb-B*) is observed. More particularly, such cancers include pancreatic, mammary and salivary carcinomas, colorectal carcinoma, exocrine pancreatic carcinoma and myeloid leukemias.

15 Examples of compounds which antagonize Raf are as follows:

(a) a compound represented by formula (I-a):



(I-a)

20

or a pharmaceutically acceptable salt thereof, wherein:

AR represents an aromatic group containing 6-10 atoms;

25

X and X' each independently represent $-(CH_2)_m-Y-(CH_2)_n-$, wherein m and n represent integers within the range of from 0 - 4, such that the sum of m and n is from 0 - 6; Y represents a member selected from the group consisting of: a direct bond; O ; $S(O)_y$, with y equal to

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0, 1 or 2; $\text{NR}^{\text{q}'}$, with $\text{R}^{\text{q}'}$ as defined below; C(O) ; OC(O) ; C(O)O ;
 $\text{SO}_x\text{NR}^{\text{q}'}$ with x equal to 1 or 2 and $\text{R}^{\text{q}'}$ as defined below; $\text{NR}^{\text{q}'}\text{SO}_x$;
 $\text{C(O)NR}^{\text{q}'}$ and $\text{NR}^{\text{q}'}\text{C(O)}$;



represents a 4 to 10 membered non-aromatic heterocycle containing at least one N atom, and optionally containing 1-2 additional N atoms and 0-1 O or S atom;

5 R^x represents H, C_{1-6} alkyl(R^{q})₃, OC_{1-6} alkyl(R^{q})₃ or C(O)C_{1-6} alkyl(R^{q})₃;

each R and R'' independently represents a member selected from the group consisting of: halo; hydroxy; C_{1-6} alkyl(R^{q})₃;
 10 OC_{1-6} alkyl(R^{q})₃; C_{3-8} cycloalkyl(R^{q})₃; CN; CONH_2 ; CONHC_{1-6} alkyl(R^{q})₃; $\text{CON}(\text{C}_{1-6}$ alkyl(R^{q})₃)₂; NH_2 ; NHC_{1-6} alkyl(R^{q})₃;
 $\text{N}(\text{C}_{1-6}$ alkyl(R^{q})₃)₂; CO_2H ; $\text{CO}_2\text{C}_{1-6}$ alkyl(R^{q})₃; C(O)C_{1-6} alkyl(R^{q})₃; aryl(R^{q})₃; heteroaryl(R^{q})₃; CF_3 ; SH; NO_2 ; $\text{SO}_y\text{C}_{1-6}$ alkyl(R^{q})₃, with y as defined above; SO_2NH_2 ; $\text{SO}_2\text{NHC}_{1-6}$ alkyl(R^{q})₃;
 15 $\text{SO}_2\text{N}(\text{C}_{1-6}$ alkyl(R^{q})₃)₂; $\text{NHSO}_2\text{C}_{1-6}$ alkyl(R^{q})₃, $\text{NHSO}_2\text{aryl}(\text{R}^{\text{q}})$ ₃, $\text{NHSO}_2\text{heteroaryl}(\text{R}^{\text{q}})$ ₃, $\text{N}(\text{R}^{\text{q}})\text{C(O)C}_{1-6}$ alkyl(R^{q})₃; $\text{NR}^{\text{q}'}\text{C(O)NH}$ (C_{1-6} alkyl(R^{q})₃);
 C_{2-4} alkenyl(R^{q})₂₋₃ and C_{2-4} alkynyl(R^{q})₁₋₃;

20 each R' independently represents a member selected from the group consisting of: CONH_2 ; CONHC_{1-6} alkyl(R^{q})₃;
 $\text{CON}(\text{C}_{1-6}$ alkyl(R^{q})₃)₂; CONHC_{3-8} cycloalkyl(R^{q})₃;
 $\text{CON}(\text{C}_{3-8}$ cycloalkyl(R^{q})₃)₂; CO_2H ; $\text{CO}_2\text{C}_{1-6}$ alkyl(R^{q})₃;
 C(O)C_{1-6} alkyl(R^{q})₃; $\text{CO}_2\text{C}_{3-8}$ cycloalkyl(R^{q})₃;
 25 C(O)C_{3-8} cycloalkyl(R^{q})₃; $-\text{[C(O)(CH}_2\text{)}_j\text{-CR}^5\text{R}^6\text{-(CH}_2\text{)}_k\text{-NR}^7\text{]}_p\text{-R}^8$;
 $-\text{C(O)C}_{3-8}$ cycloalkyl(R^{q})₃; $-\text{C(O)heterocyclyl}(\text{R}^{\text{q}})$ ₃; $\text{CON}[\text{C}_{1-6}$ alkyl(R^{q})₃][C_{3-8} cycloalkyl(R^{q})₃]; $\text{C(O)aryl}(\text{R}^{\text{q}})$ ₃ ,
 $\text{C(O)heteroaryl}(\text{R}^{\text{q}})$ ₃ ;

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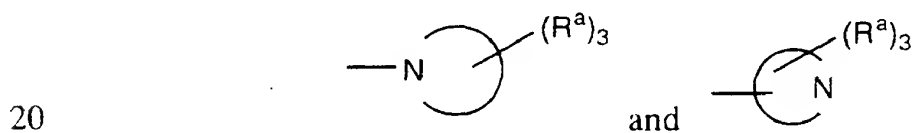
wherein p represents 1, 2 or 3;

j and k are integers independently selected from 0 - 3;

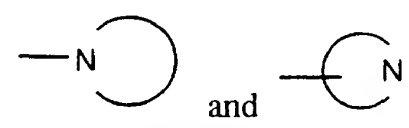
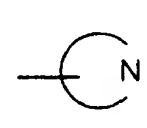
each R^5 and R^6 independently represents H, aryl, C_{1-6} alkyl(R^q)₃, or each CR^5R^6 taken in combination represents a 3, 4, 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or a heteroaryl group, wherein when p equals 1, at least one of j and k is 1, 2 or 3;

each R^7 and R^8 independently represents H, C_{1-6} alkyl or aryl;

R^q represents a member selected from the group consisting of: R^q ; CN; CO_2H ; CO_2C_{1-4} alkyl; $C(O)C_{1-4}$ alkyl; aryl(R^a)₃; NH_2 ; NHC_{1-6} alkyl(R^a)₃; $N(C_{1-6}$ alkyl(R^a)₃)₂; heteroaryl(R^a)₃; $CONH_2$; SH; $S(O)_y C_{1-6}$ alkyl(R^a)₃; $C(O)NHC_{1-6}$ alkyl(R^a)₃; $C(O)N(C_{1-6}$ alkyl(R^a)₃)₂; -heteroalkyl(R^a)₃; - $NHC(O)NH_2$; - $NHC(NH)NH_2$;



wherein

25  and  independently represent mono or bicyclic ring systems, non-aromatic or partially aromatic, containing from 5-10 ring atoms, 1-4 of which are N and 0-1 of which are O or $S(O)_y$, with y equal to 0, 1 or 2, optionally containing 1-2 carbonyl groups;

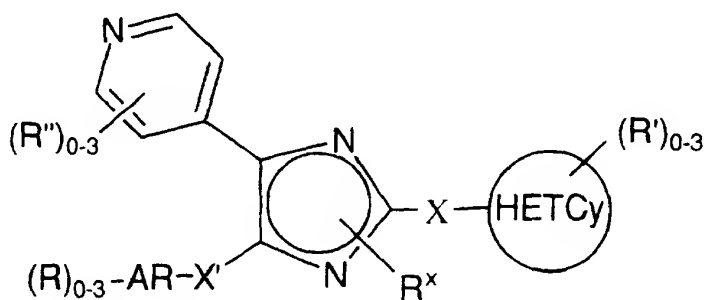
30 each R^a independently represents a member selected from the group consisting of: H, C_{1-6} alkyl, OC_{1-6} alkyl, aralkyl, substituted aralkyl, heteroaralkyl, substituted heteroaralkyl, aralkoxy, substituted

-8-

- aralkoxy, halo, hydroxy, CN, CONH₂, CONHC₁₋₆ alkyl, CON(C₁₋₆ alkyl)₂, CO₂H, CO₂C₁₋₆ alkyl, C(O)C₁₋₆ alkyl, phenyl, CF₃, SH, NO₂, SO_yC₁₋₆ alkyl, with y as defined above; SO₂NH₂, SO₂NHC₁₋₆ alkyl, NHSO₂(substituted aryl), NHSO₂(substituted heteroaryl),
- 5 NHSO₂C₁₋₆alkyl, NHSO₂aryl, NHSO₂heteroaryl, NH₂, NHC₁₋₆ alkyl, N(C₁₋₆ alkyl)₂, NHC(O)C₁₋₆ alkyl, NHC(O)NH(C₁₋₆ alkyl), C₂₋₄ alkenyl and C₂₋₄ alkynyl;

and R^{q'} represents H, OH, C₁₋₄ alkyl, -OC₁₋₄ alkyl, aryl
 10 or C(O)C₁₋₄ alkyl;

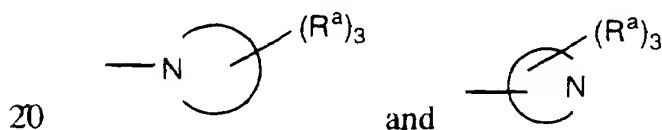
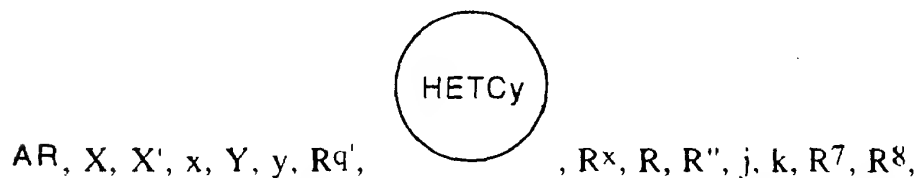
(b) a compound represented by formula (I-b)



(I-b)

15

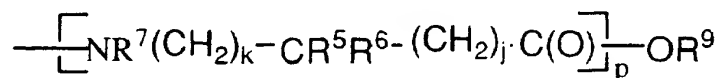
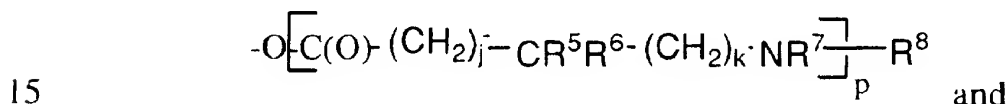
or a pharmaceutically acceptable salt thereof, wherein:



are as defined above with respect to formula (I-a);

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- each R' independently represents a member selected from the group consisting of: hydroxy; C₁₋₆ alkyl(R^q)₃; C₃₋₈ cycloalkyl(R^q)₃; OC₁₋₆ alkyl(R^q)₃; OC₃₋₈ cycloalkyl(R^q)₃; heterocyclyl(R^q)₃; CN; NH(R^q"); NHC₁₋₆ alkyl(R^q)₃; N(C₁₋₆ alkyl(R^q)₃)₂; NHC₃₋₈ cycloalkyl(R^q)₃; N(C₃₋₈ cycloalkyl(R^q)₃)₂; CF₃; SH; NO₂; C₂₋₄ alkenyl(R^q)₂₋₃, aryl(R^q)₃, heteroaryl(R^q)₃; C₂₋₄ alkynyl(R^q)₁₋₃; -OC(O) C₃₋₈ cycloalkyl(R^q)₃; SO₂NH₂; SO₂NHC₁₋₆ alkyl(R^q)₃; SO₂N(C₁₋₆ alkyl(R^q)₃)₂; NHSO₂C₁₋₆ alkyl(R^q)₃, NHSO₂aryl(R^q)₃, NHSO₂heteroaryl(R^q)₃,
 10 -OC(O)heterocyclyl(R^q)₃; N(R^q)C(O)C₁₋₆ alkyl(R^q)₃; NR^qC(O)NH(C₁₋₆ alkyl(R^q)₃); -OC(O)C₁₋₆ alkyl(R^q)₃; -OC(O)aryl(R^q)₃, -OC(O)heteroaryl(R^q)₃; -C(=NR^q)NH₂; -C(=N^q)NHC₁₋₆ alkyl(R^q)₃, -C(=N^q)N(C₁₋₆ alkyl(R^q)₃)₂;



- R⁵ and R⁶ are independently H, aryl, C₁₋₆ alkyl(R^q)₃, or
 20 CR⁵R⁶ in combination represents a 3, 4, 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or a heteroaryl group;

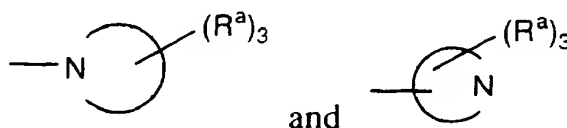
- p represents 1, 2 or 3, with the proviso that when p represents 1, CR⁵R⁶ represents a 3, 4, 5 or 6 membered cycloalkyl
 25 group or a heterocyclyl group, an aryl group or a heteroaryl group, and at least one of j and k is 1, 2 or 3;

- R⁹ represents H, a negative charge balanced by a positively charged group or a protecting group;
 30

R^q represents a member selected from the group consisting of: R^q'; CN; CO₂H; CO₂C₁₋₄ alkyl; C(O)C₁₋₄ alkyl; NH(R^q"); aryl(R^a)₃; heteroaryl(R^a)₃; NHC₁₋₄ alkyl; N(C₁₋₄ alkyl)₂; CONH₂;

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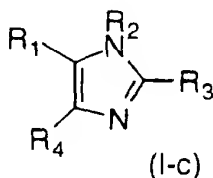
SH; S(O)_y C₁₋₆ alkyl(R^a)₃; C(O)NHC₁₋₆ alkyl(R^a)₃; C(O)N(C₁₋₆ alkyl(R^a)₃)₂; NHC(NH)NH₂; -heteroalkyl(R^a)₃; -NHC(O)NH₂;



5

and R^q represents H, OH or OC₁₋₄ alkyl,

and (c) a compound represented by formula (I-c):



10

or a pharmaceutically acceptable salt thereof,
wherein:

15 R₁ is 4-pyridyl, pyrimidinyl, quinazolin-4-yl, quinolyl, isoquinolinyl, 1-imidazolyl or 1-benzimidazolyl which is optionally substituted with one or two substituents each of which is independently selected from C₁₋₄ alkyl, halogen, C₁₋₄ alkoxy, C₁₋₄ alkylthio, NR₁₀R₂₀, or N-heterocyclyl ring which ring has from 5 to 7 members and optionally contains an additional heteroatom selected from oxygen, sulfur or
20 NR₂₂;

R₂ is hydrogen, -(CR₁₀R₂₀)_n OR₁₂, heterocyclyl, heterocyclyl C₁₋₁₀ alkyl, C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇ cycloalkyl, C₃₋₇ cycloalkyl C₁₋₁₀ alkyl, C₅₋₇ cycloalkenyl, aryl, aryl C₁₋₁₀ alkyl, heteroaryl, heteroaryl
25 C₁₋₁₀ alkyl, (CR₁₀R₂₀)_n OR₁₃, (CR₁₀R₂₀)_n S(O)_m R₂₅, (CR₁₀R₂₀)_n NHS(O)₂ R₂₅, (CR₁₀R₂₀)_n NR₈R₉, (CR₁₀R₂₀)_n NO₂, (CR₁₀R₂₀)_n CN, (CR₁₀R₂₀)_n S(O)_m NR₈R₉, (CR₁₀R₂₀)_n C(Z) R₁₃, (CR₁₀R₂₀)_n C(Z) OR₁₃, (CR₁₀R₂₀)_n NR₁₀ C(Z) NR₈R₉, (CR₁₀R₂₀)_n C(Z) NR₁₃ OR₁₂,
30 (CR₁₀R₂₀)_n NR₁₀ C(Z) R₁₃, (CR₁₀R₂₀)_n NR₁₀ C(Z) NR₈R₉,

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- (CR₁₀R₂₀)_n'N(OR₂₁)C(Z)NR₈R₉, (CR₁₀R₂₀)_n'N(OR₂₁)C(Z)R₁₃,
 (CR₁₀R₂₀)_n'C(=NOR₂₁)R₁₃, (CR₁₀R₂₀)_n'NR₁₀C(=NR₂₇)NR₈R₉,
 (CR₁₀R₂₀)_n'OC(Z)NR₈R₉, (CR₁₀R₂₀)_n'NR₁₀C(Z)NR₈R₉,
 (CR₁₀R₂₀)_n'C(Z)OR₁₀, 5-(R₂₅)-1,2,4-oxadiazol-3-yl or 4-(R₁₂)-
 5-(R₁₈R₁₉)-4,5-dihydro-1,2,4-oxadiazol-3-yl; wherein the aryl,
 arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl or
 heterocyclalkyl moieties may be optionally substituted;
 n' is an integer having a value of 1 to 10;
 m is 0 or the integer 1 or 2;
- 10 R₃ is Q-(Y₁)_t;
 Q is an aryl or heteroaryl group;
 t is a number having a value of 1, 2 or 3;
 Z is oxygen or sulfur;
 n is 0 or an integer from 1 to 10;
- 15 Y₁ is independently selected from hydrogen, C₁₋₅ alkyl, halo-substituted C₁₋₅ alkyl, halogen, or -(CR₁₀R₂₀)_nY₂;
 Y₂ is -OR₈, -NO₂, -S(O)_m'R₁₁, -SR₈, -S(O)_m'OR₈, -S(O)_mNR₈R₉,
 -NR₈R₉, -O(CR₁₀R₂₀)_nNR₈R₉, -C(O)R₈, -CO₂R₈,
 -CO₂(CR₁₀R₂₀)_nCONR₈R₉, -ZC(O)R₈, -CN, -C(Z)NR₈R₉,
 20 NR-NR₁₀C(Z)R₈, -C(Z)NR₈OR₉, -NR₁₀C(Z)NR₈R₉,
 -NR₁₀S(O)_mR₁₁, -N(OR₂₁)C(Z)NR₈R₉, -N(OR₂₁)C(Z)R₈,
 -C(=NOR₂₁)R₈, -NR₁₀C(=NR₁₅)SR₁₁, -NR₁₀C(=NR₁₅)NR₈R₉,
 -NR₁₀C(=CR₁₄R₂₄)SR₁₁, -NR₁₀C(=CR₁₄R₂₄)NR₈R₉,
 -NR₁₀C(O)C(O)NR₈R₉, -NR₁₀C(O)C(O)OR₁₀,
 25 -C(=NR₁₃)NR₈R₉, -C(=NOR₁₃)NR₈R₉, -C(=NR₁₃)ZR₁₁,
 -OC(Z)NR₈R₉, -NR₁₀S(O)_mCF₃, -NR₁₀C(Z)OR₁₀, 5-(R₁₈)-
 1,2,4-oxadiazol-3-yl or 4-(R₁₂)-5-(R₁₈R₁₉)-4,5-dihydro-1,2,4-
 oxadiazol-3-yl;
 m' is a number having a value of 1 or 2;
- 30 R₄ is phenyl, naphth-1-yl or naphth-2-yl which is optionally substituted
 by one or two substituents, each of which is independently selected,
 and which, for a 4-phenyl, 4-naphth-1-yl or 5-naphth-1-yl
 substituent, is halo, cyano, -C(Z)NR₇R₁₇, -C(Z)OR₂₃,
 -(CR₁₀R₂₀)_m'COR₃₆, SR₅, -SOR₅, OR₃₆, halo-substituted-C₁₋₄

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- alkyl, C₁₋₄ alkyl, -ZC(Z)R₃₆, -NR₁₀C(Z)R₂₃ or
 -(CR₁₀R₂₀)^{m''}NR₁₀R₂₀ and which, for other positions of
 substitution, is halo, cyano, -C(Z)NR₁₆R₂₆, -C(Z)OR₈,
 -(CR₁₀R₂₀)^{m''}COR₈, -S(O)_mR₈, -OR₈, halo-substituted-C₁₋₄
 5 alkyl, C₁₋₄ alkyl, -(CR₁₀R₂₀)^{m''}NR₁₀C(Z)R₈, -NR₁₀S(O)_{m'}R₁₁,
 -NR₁₀S(O)_{m'}NR₇R₁₇, -ZC(Z)R₈ or -(CR₁₀R₂₀)^{m'}NR₁₆R₂₆;
 wherein m'' is 0 to 5 and m''' is 0 or 1;
 R₅ is hydrogen, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl or NR₇R₁₇,
 excluding the moieties -SR₅ being -SNR₇R₁₇ and -SOR₅ being
 10 -SOH;
 R₆ is C₁₋₄ alkyl, halo-substituted-C₁₋₄ alkyl, C₁₋₄ alkenyl, C₂₋₄
 alkynyl or C₃₋₅ cycloalkyl;
 R₇ and R₁₇ are each independently selected from hydrogen or C₁₋₄
 alkyl, or R₇ and R₁₇ together with the nitrogen to which they are
 15 attached form a heterocyclic ring of 5 to 7 members which ring
 optionally contains an additional heteroatom selected from oxygen,
 sulfur or NR₂₂;
 R₈ is hydrogen, heterocyclyl, heterocyclylalkyl or R₁₁;
 R₉ is hydrogen, C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇
 20 cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylalkyl, heteroaryl or
 heteroarylalkyl or R₈ and R₉ may together with the nitrogen to
 which they are attached form a heterocyclic ring of 5 to 7 members
 which ring optionally contains an additional heteroatom selected
 from oxygen, sulfur or NR₁₂;
 25 R₁₀ and R₂₀ are each independently selected from hydrogen and C₁₋₄
 alkyl;
 R₁₁ is C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀
 alkynyl, C₃₋₇ cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylalkyl,
 heteroaryl or heteroarylalkyl;
 30 R₁₂ is hydrogen, -C(Z)R₁₃ or optionally substituted C₁₋₄ alkyl,
 optionally substituted arylC₁₋₄ alkyl or S(O)₂R₂₅;
 R₁₃ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl,
 heterocyclyl C₁₋₁₀ alkyl, aryl, aryl C₁₋₁₀ alkyl, heteroaryl or
 heteroaryl C₁₋₁₀ alkyl;

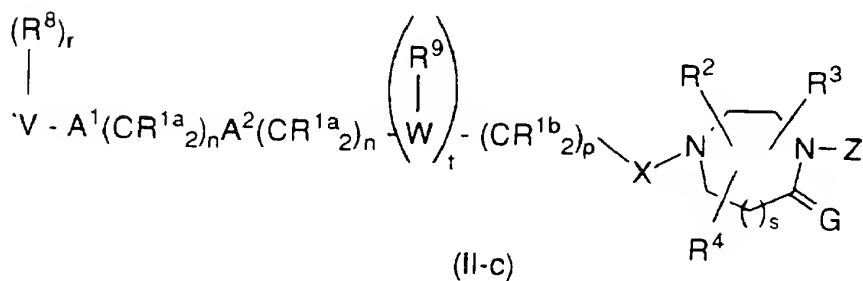
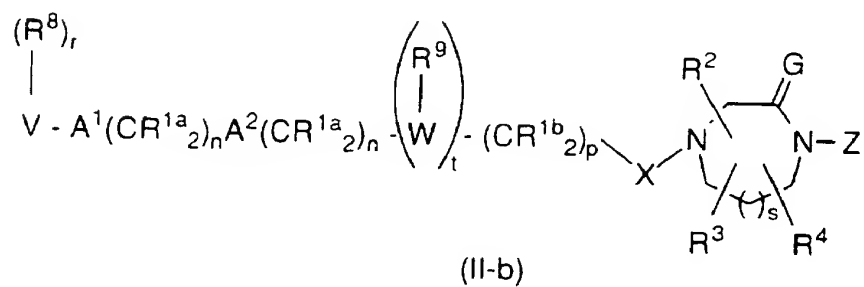
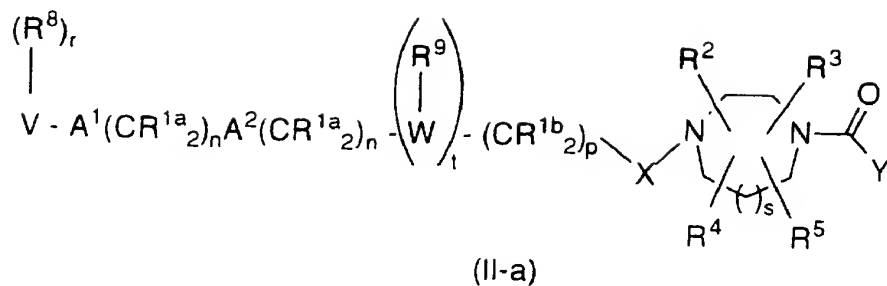
-13-

- R₁₄ and R₂₄ is each independently selected from hydrogen, alkyl, nitro or cyano;
- R₁₅ is hydrogen, cyano, C₁₋₄ alkyl, C₃₋₇ cycloalkyl or aryl;
- 5 R₁₆ and R₂₆ is each independently selected from hydrogen or optionally substituted C₁₋₄ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl, or together with the nitrogen which they are attached form a heterocyclic ring of 5 to 7 members which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₁₂;
- 10 R₁₈ and R₁₉ is each independently selected from hydrogen, C₁₋₄ alkyl, substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl or together denote a oxygen or sulfur;
- R₂₁ is hydrogen, a pharmaceutically acceptable cation, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, aryl, aryl C₁₋₄ alkyl, heteroaryl, heteroarylalkyl, 15 heterocyclyl, aroyl, or C₁₋₁₀ alkanoyl;
- R₂₂ is R₁₀ or C(Z)-C₁₋₄ alkyl;
- R₂₃ is C₁₋₄ alkyl, halo-substituted-C₁₋₄ alkyl or C₃₋₅ cycloalkyl;
- R₃₆ is hydrogen or R₂₃;
- R₂₅ is C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl, aryl, arylalkyl, 20 heterocyclyl, heterocyclyl-C₁₋₁₀ alkyl, heteroaryl or heteroarylalkyl;
- R₂₇ is hydrogen, cyano, C₁₋₄ alkyl, C₃₋₇ cycloalkyl or aryl; or a pharmaceutically acceptable salt thereof.

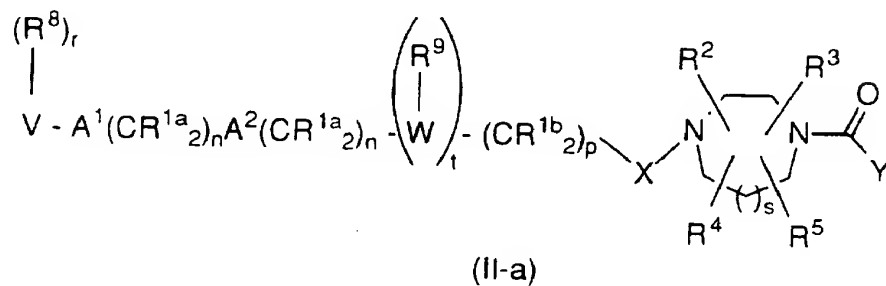
Examples of farnesyl protein transferase inhibiting 25 compounds include the following:

- (a) a compound represented by formula (II-a) through (II-c):

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wherein with respect to formula (II-a):



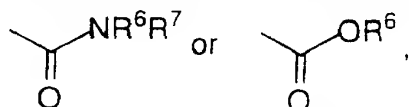
or a pharmaceutically acceptable salt thereof,

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R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)-NR¹⁰-;

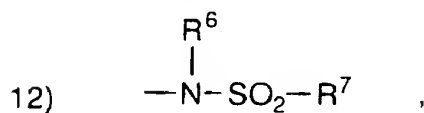
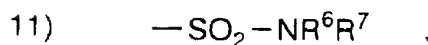
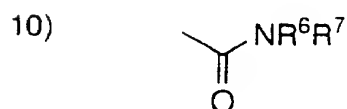
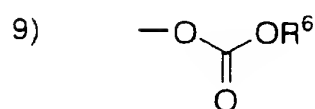
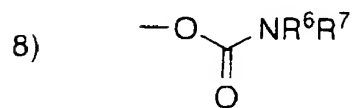
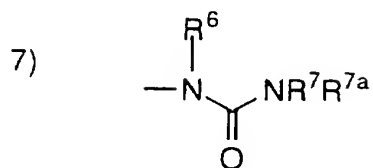
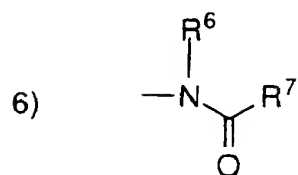
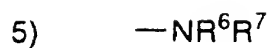
R² and R³ are independently selected from: H; unsubstituted or substituted C₁-8 alkyl, unsubstituted or substituted C₂-8 alkenyl, unsubstituted or substituted C₂-8 alkynyl, unsubstituted or substituted aryl, unsubstituted or substituted heterocycle,



wherein the substituted group is substituted with one or more of:

- 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁-4 alkyl,
 - b) (CH₂)_pOR⁶,
 - c) (CH₂)_pNR⁶R⁷,
 - d) halogen,
- 2) C₃-6 cycloalkyl,
- 3) OR⁶,
- 4) SR⁶, S(O)R⁶, SO₂R⁶,

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5 R^2 and R^3 are attached to the same C atom and are combined to form $(\text{CH}_2)_u$ - wherein one of the carbon atoms is optionally replaced by a
 10 moiety selected from: O, S(O)_m , —NC(O)— , and $\text{—N(COR}^{10}\text{)—}$;

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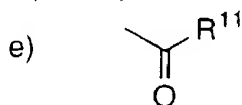
R^4 and R^5 are independently selected from H and CH_3 ;

5 and any two of R^2 , R^3 , R^4 and R^5 are optionally attached to the same carbon atom;

R^6 , R^7 and R^{7a} are independently selected from: H; C_{1-4} alkyl, C_{3-6} cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with:

10

- a) C_{1-4} alkoxy,
- b) aryl or heterocycle,
- c) halogen,
- d) HO,



f) $-SO_2R^{11}$, or

15

g) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^7 and R^{7a} may be joined in a ring;

20

R^8 is independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 , $R^{10}_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and

25

- c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$,

30

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$R^{10}C(O)NH-$, CN , $H_2N-C(NH)-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{10}OC(O)NH-$;

R^9 is selected from:

- 5 a) hydrogen,
- b) C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , NO_2 , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 10 c) C_1-C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

- 15 R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

- 20 A^1 and A^2 are independently selected from: a bond, $-CH=CH-$, $-C\equiv C-$, $-C(O)-$, $-C(O)NR^{10}-$, $-NR^{10}C(O)-$, O, $-N(R^{10})-$, $-S(O)_2N(R^{10})-$, $-N(R^{10})S(O)_2-$, or $S(O)_m$;

V is selected from:

- 25 a) hydrogen,
- b) heterocycle,
- c) aryl,
- d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N,
- 30 and
- e) C_2-C_{20} alkenyl,

provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

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W is a heterocycle;

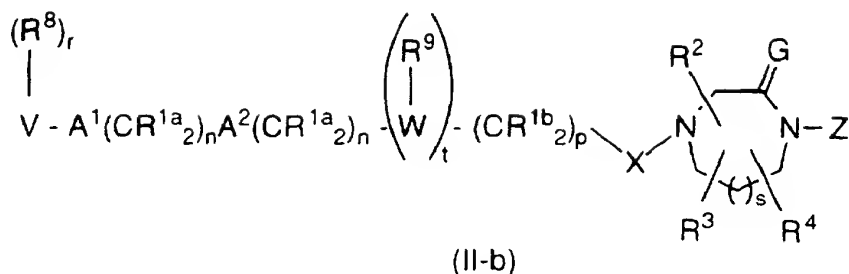
X is $-\text{CH}_2-$, $-\text{C}(=\text{O})-$, or $-\text{S}(=\text{O})_m-$;

5 Y is aryl, heterocycle, unsubstituted or substituted with one or more of:

- 1) C_{1-4} alkyl, unsubstituted or substituted with:
 - a) C_{1-4} alkoxy,
 - b) NR^6R^7 ,
 - 10 c) C_{3-6} cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO ,
 - f) $-\text{S}(\text{O})_m\text{R}^6$, or
 - g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$,
 - 15 2) aryl or heterocycle,
 - 3) halogen,
 - 4) OR^6 ,
 - 5) NR^6R^7 ,
 - 6) CN ,
 - 20 7) NO_2 ,
 - 8) CF_3 ;
 - 9) $-\text{S}(\text{O})_m\text{R}^6$,
 - 10) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or
 - 11) $\text{C}_3\text{-C}_6$ cycloalkyl;
- 25
- m is 0, 1 or 2;
- n is 0, 1, 2, 3 or 4;
- p is 0, 1, 2, 3 or 4;
- r is 0 to 5, provided that r is 0 when V is hydrogen;
- 30 s is 0 or 1;
- t is 0 or 1; and
- u is 4 or 5;

with respect to formula (II-b):

-20-



or a pharmaceutically acceptable salt thereof,

5 R^{1a} , R^{1b} , R^{10} , R^{11} , m , R^2 , R^3 , R^6 , R^7 , p , R^{7a} , u , R^8 , A^1 , A^2 , V , W , X , n , p , r , s , t and u are as defined above with respect to formula (II-a);

R^4 is selected from H and CH_3 ;

10 and any two of R^2 , R^3 and R^4 are optionally attached to the same carbon atom;

R^9 is selected from:

- a) hydrogen,
- 15 b) alkenyl, alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 20 c) C_1 - C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

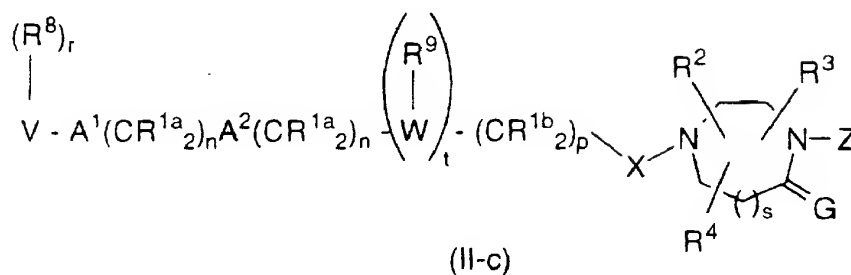
G is H_2 or O;

25- Z is aryl, heteroaryl, arylmethyl, heteroarylmethyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more of the following:

- 1) C_1 -4 alkyl, unsubstituted or substituted with:
 - a) C_1 -4 alkoxy,

- | | |
|----|---|
| | b) NR^6R^7 , |
| | c) C_{3-6} cycloalkyl, |
| | d) aryl or heterocycle, |
| | e) HO , |
| 5 | f) $-\text{S}(\text{O})_m\text{R}^6$, or |
| | g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, |
| | 2) aryl or heterocycle, |
| | 3) halogen, |
| | 4) OR^6 , |
| 10 | 5) NR^6R^7 , |
| | 6) CN , |
| | 7) NO_2 , |
| | 8) CF_3 ; |
| | 9) $-\text{S}(\text{O})_m\text{R}^6$, |
| 15 | 10) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or |
| | 11) $\text{C}_3\text{-C}_6$ cycloalkyl; |

with respect to formula (II-c):



- 20 or a pharmaceutically acceptable salt thereof,

$R^{1a}, R^{1b}, R^{10}, R^{11}, m, R^2, R^3, R^6, R^7, p, u, R^{7a}, R^8, A^1, A^2, V, W, X,$
 n, r and t are as defined above with respect to formula (II-a);

- 25- R⁴ is selected from H and CH₃;

and any two of R², R³ and R⁴ are optionally attached to the same carbon atom;

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G is O;

5 Z is aryl, heteroaryl, arylmethyl, heteroarylmethyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more of the following:

- 1) C₁₋₄ alkyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,
 - 10 c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁶, or
 - g) -C(O)NR⁶R⁷,
- 15 2) aryl or heterocycle,
- 3) halogen,
- 4) OR⁶,
- 5) NR⁶R⁷,
- 6) CN,
- 20 7) NO₂,
- 8) CF₃;
- 9) -S(O)_mR⁶,
- 10) -C(O)NR⁶R⁷, or
- 11) C₃₋₆ cycloalkyl;

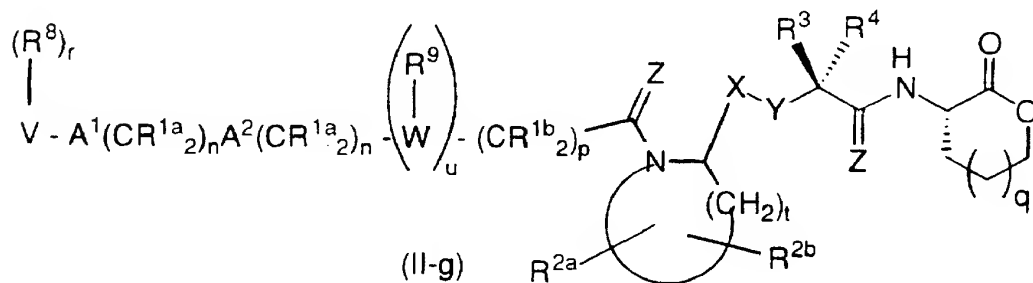
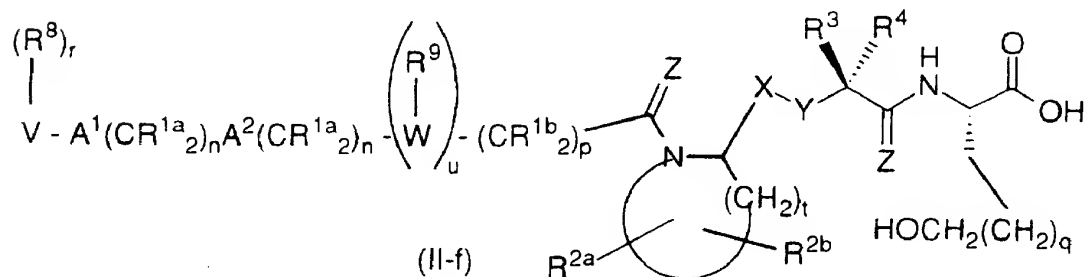
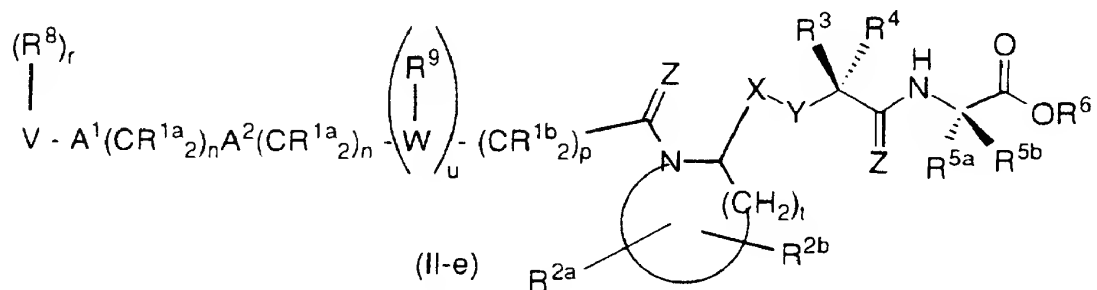
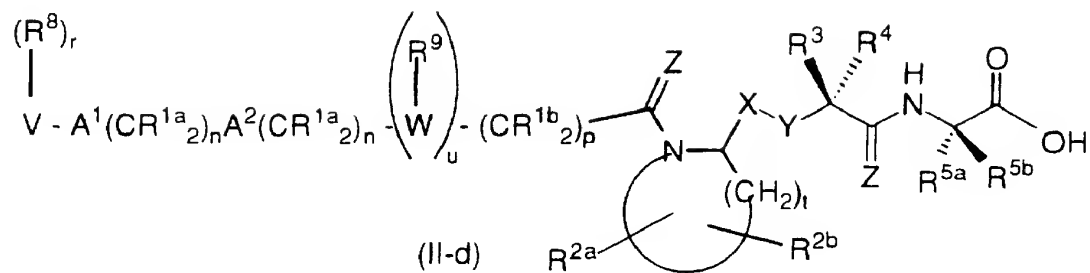
25

and

s is I;

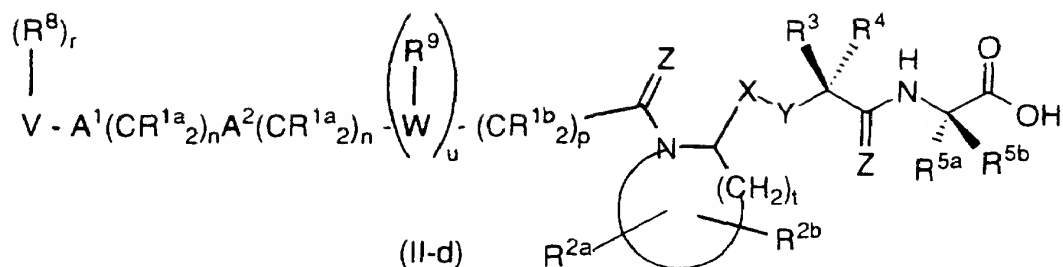
30 (b) a compound represented by formula (II-d) through (II-g):

-23-



wherein with respect to formula (II-d):

-24-



or a pharmaceutically acceptable salt thereof,

R^{11} , V , W , m , n , p and r are as defined above with respect to formula
 5 (II-a);

R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- 10 b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- 15 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;

20

R^{2a} and R^{2b} are independently selected from:

- a) hydrogen,
- 25 b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N₃, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃,

-25-

- N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

5

R³ and R⁴ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
- 10 i) methionine sulfoxide, or
- ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
- 15 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or
- 20 substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

R³ and R⁴ are combined to form - (CH₂)_s - ;

25 R^{5a} and R^{5b} are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
- 30 i) methionine sulfoxide, or
- ii) methionine sulfone,
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
- wherein the substituent is selected from F, Cl, Br, CF₃, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-,

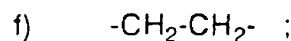
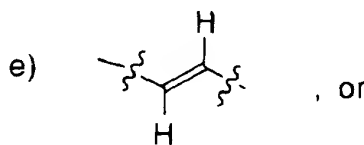
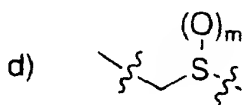
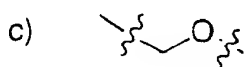
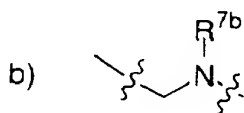
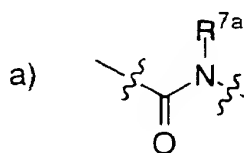
-26-

$R^{10}C(O)NR^{10}$ -, CN , $(R^{10})_2N-C(NR^{10})$ -, $R^{10}C(O)$ -,
 $R^{10}OC(O)$ -, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}$ - and
 C_1 - C_{20} alkyl,

- 5 d) C_1 - C_6 alkyl substituted with an unsubstituted or
substituted group selected from aryl, heterocycle and
 C_3 - C_{10} cycloalkyl; or

R^{5a} and R^{5b} are combined to form $-(CH_2)_s-$ wherein one of the
carbon atoms is optionally replaced by a moiety selected from: O,
10 $S(O)_m$, $-NC(O)-$, and $-N(COR^{10})-$:

X-Y is



15 R^{7a} is selected from

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- 5
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
 - e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- 10
- a) hydrogen,
 - b) unsubstituted or substituted aryl,
 - c) unsubstituted or substituted heterocycle,
 - d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 - e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
 - f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
 - g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;
- 15
- 20
- 25

R⁸ is independently selected from:

- 30
- a) hydrogen,
 - b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

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- 5 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;

R⁹ is selected from:

- 10 a) hydrogen,
 b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
 15 c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

20 R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-, -N(R¹⁰)S(O)₂-, or S(O)_m;

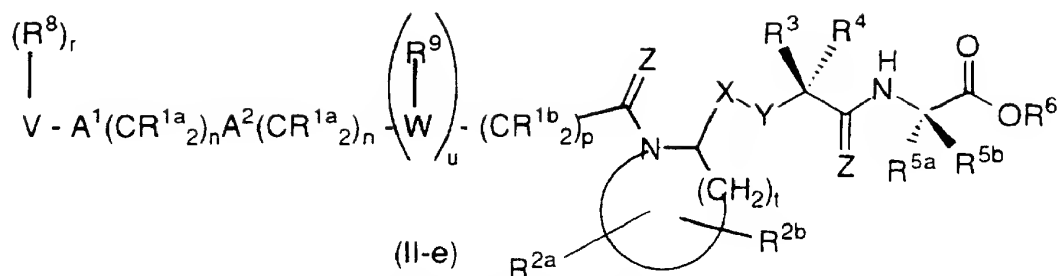
25 Z is independently H₂ or O;

s is 4 or 5;
 t is 3, 4 or 5; and
 u is 0 or 1;

30

with respect to formula (II-e):

-29-



or a pharmaceutically acceptable salt thereof,

5 R^{11} , W, m, n, p and r are as defined above with respect to formula (II-a);

R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- 10 b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- 15 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;

R^{2a} and R^{2b} are independently selected from:

- 20 a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N₃, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- 25 c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, $R^{10}O$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and

-30-

- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

5 R³ and R⁴ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 10 ii) methionine sulfone.
 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,
 15 CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 and
 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and
 20 C₃-C₁₀ cycloalkyl; or

R³ and R⁴ are combined to form - (CH₂)_s - ;

R^{5a} and R^{5b} are independently selected from:

- 25 a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 ii) methionine sulfone,
 30 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
 wherein the substituent is selected from F, Cl, Br, CF₃, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-.

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$R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and
 C_1-C_{20} alkyl, and

- d) C_1-C_6 alkyl substituted with an unsubstituted or
substituted group selected from aryl, heterocycle and
 C_3-C_{10} cycloalkyl; or

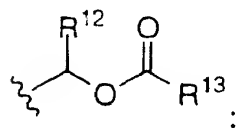
R^{5a} and R^{5b} are combined to form $-(CH_2)_s-$ wherein one of the
carbon atoms is optionally replaced by a moiety selected from: O,
 $S(O)_m$, $-NC(O)-$, and $-N(COR^{10})-$;

R^6 is

- a) substituted or unsubstituted C_1-C_8 alkyl, substituted or
unsubstituted C_5-C_8 cycloalkyl, or substituted or
unsubstituted cyclic amine, wherein the substituted alkyl,
cycloalkyl or cyclic amine is substituted with 1 or 2
substituents independently selected from:

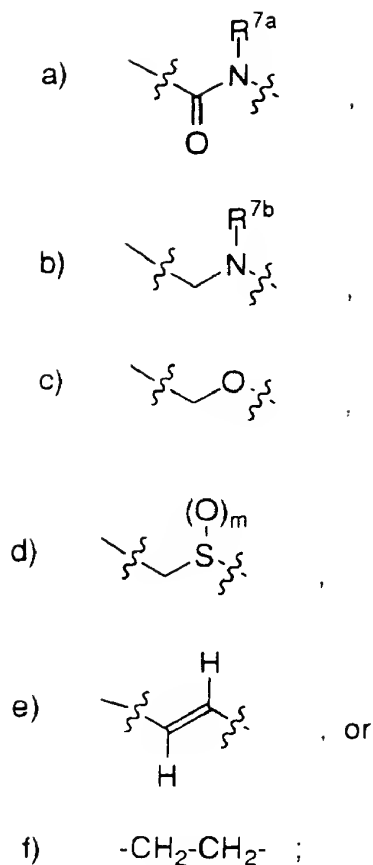
- 1) C_1-C_6 alkyl,
- 2) aryl,
- 3) heterocycle,
- 4) $-N(R^{11})_2$,
- 5) $-OR^{10}$, or

b)



$X-Y$ is

-32-

R^{7a} is selected from

- 5 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
 10 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted
 or substituted group selected from aryl, heterocycle and
 C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- 15 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl.

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- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
 - 5 f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
 - 10 g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;
- 15 R⁸ is independently selected from:
- a) hydrogen,
 - b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
 - 20 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;
 - 25

R⁹ is selected from:

- a) hydrogen,
- 30 b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

-34-

- c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

5

R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

R¹² is hydrogen or C₁-C₆ alkyl;

10

R¹³ is C₁-C₆ alkyl;

A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-,

15

-N(R¹⁰)S(O)₂-, or S(O)_m;

Z is independently H₂ or O;

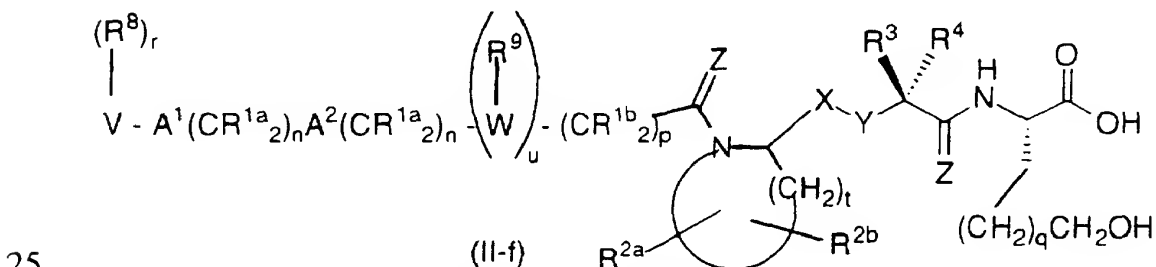
s is 4 or 5;

20

t is 3, 4 or 5; and

u is 0 or 1;

with respect to formula (II-f):



or a pharmaceutically acceptable salt thereof.

R¹¹, V, W, m, n, p and r are as defined above with respect to formula (II-a);

-35-

R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl,
C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN,
5 NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃,
-N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-,
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl,
heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆
alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN,
10 (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃,
-N(R¹⁰)₂, or R¹¹OC(O)-NR¹⁰-;

R^{2a} and R^{2b} are independently selected from:

- a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆
15 alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃,
(R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or
R¹¹OC(O)NR¹⁰-,
- c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆
20 alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂,
(R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃,
-N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or
25 substituted group selected from aryl, heterocyclyl and
C₃-C₁₀ cycloalkyl;

R³ and R⁴ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring
30 amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl,
C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

-36-

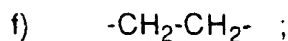
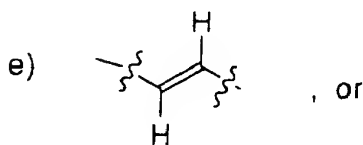
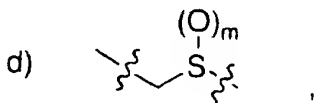
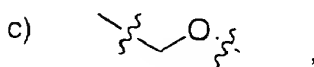
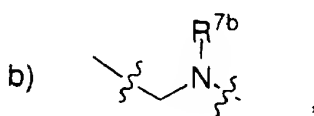
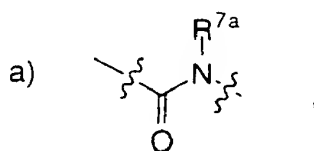
wherein the substituent is selected from F, Cl, Br,
 $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$,
 CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$,
 N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1-C_{20} alkyl,
 and

5

- d) C_1-C_6 alkyl substituted with an unsubstituted or
 substituted group selected from aryl, heterocycle and
 C_3-C_{10} cycloalkyl; or

10 R^3 and R^4 are combined to form $-(CH_2)_s-$;

X-Y is



R^{7a} is selected from

- 15 a) hydrogen,

-37-

- 5 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C3-C10 cycloalkyl, and
 e) C1-C6 alkyl substituted with hydrogen or an unsubstituted
 or substituted group selected from aryl, heterocycle and
 C3-C10 cycloalkyl;

R^{7b} is selected from

- 10 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C3-C10 cycloalkyl,
 e) C1-C6 alkyl substituted with hydrogen or an unsubstituted
 or substituted group selected from aryl, heterocycle and
 15 C3-C10 cycloalkyl,
 f) a carbonyl group which is bonded to an unsubstituted or
 substituted group selected from aryl, heterocycle, C3-C10
 cycloalkyl and C1-C6 alkyl substituted with hydrogen or an
 unsubstituted or substituted group selected from aryl,
 20 heterocycle and C3-C10 cycloalkyl, and
 g) a sulfonyl group which is bonded to an unsubstituted or
 substituted group selected from aryl, heterocycle, C3-C10
 cycloalkyl and C1-C6 alkyl substituted with hydrogen or an
 unsubstituted or substituted group selected from aryl,
 25 heterocycle and C3-C10 cycloalkyl;

R⁸ is independently selected from:

- 30 a) hydrogen,
 b) aryl, heterocycle, C3-C10 cycloalkyl, C2-C6 alkenyl,
 C2-C6 alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-,
 R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-,
 R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or
 R¹¹OC(O)NR¹⁰-, and

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- 5 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;

R⁹ is selected from:

- 10 a) hydrogen,
 b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
 15 c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

20 R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

R¹² is hydrogen or C₁-C₆ alkyl;

R¹³ is C₁-C₆ alkyl;

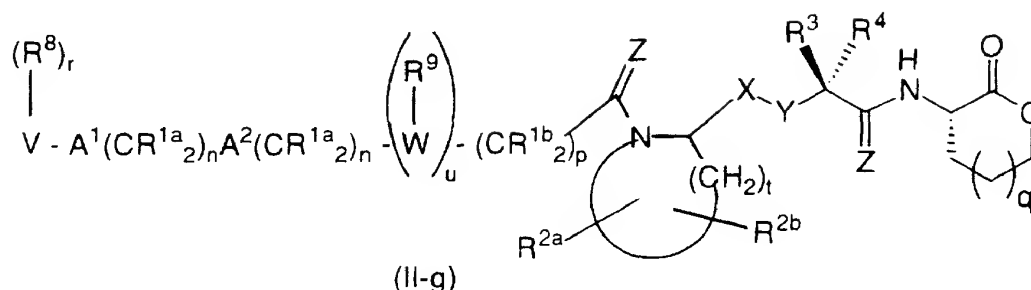
25 A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-, -N(R¹⁰)S(O)₂-, or S(O)_m;

30 Z is independently H₂ or O;

q is 0, 1 or 2;
 s is 4 or 5;
 t is 3, 4 or 5; and
 u is 0 or 1;

-39-

with respect to formula (II-g):



or a pharmaceutically acceptable salt thereof.

5

R^{11} , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

R^{1a} and R^{1b} are independently selected from:

10

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;

20

R^{2a} and R^{2b} are independently selected from:

- a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N₃, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆

25

-40-

alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , NO_2 , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}-$, and

- 5 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C_3 - C_{10} cycloalkyl;

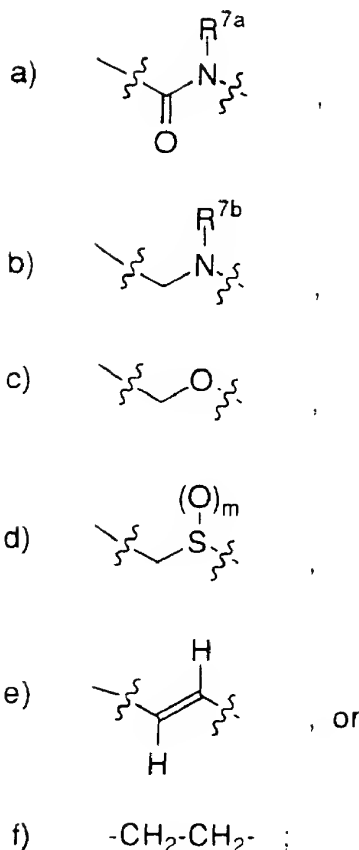
R^3 and R^4 are independently selected from:

- 10 a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 ii) methionine sulfone,
 15 c) substituted or unsubstituted C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_3 - C_{10} cycloalkyl, aryl or heterocycle group,
 wherein the substituent is selected from F , Cl , Br , $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl,
 20 and
 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

- 25 R^3 and R^4 are combined to form $-(CH_2)_s-$;

-41-

X-Y is

R^{7a} is selected from

- 5 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
 10 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted
 or substituted group selected from aryl, heterocycle and
 C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- 15 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle.

-42-

- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
- 5 f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
- 10 g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

15

R⁸ is independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-,
 20 R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-,
 R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or
 R¹¹OC(O)NR¹⁰-, and
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl,
 heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆
 25 alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-,
 R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-,
 N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;

R⁹ is selected from:

- 30 a) hydrogen,
- b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl,
 Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂,
 (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃,
 -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

-43-

- c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

5

R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

R¹² is hydrogen or C₁-C₆ alkyl;

10

R¹³ is C₁-C₆ alkyl;

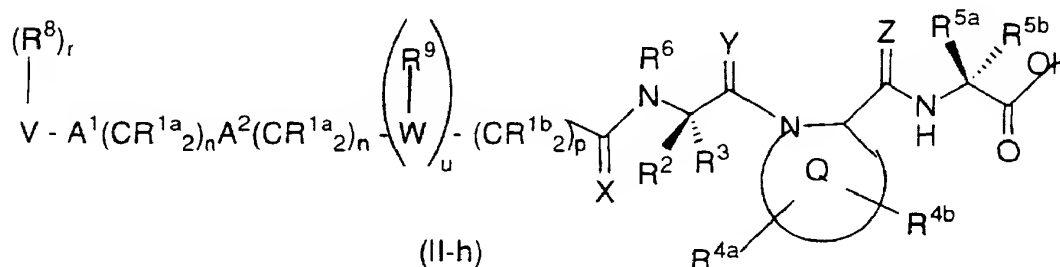
A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-,
15 -N(R¹⁰)S(O)₂-, or S(O)_m;

Z is independently H₂ or O;

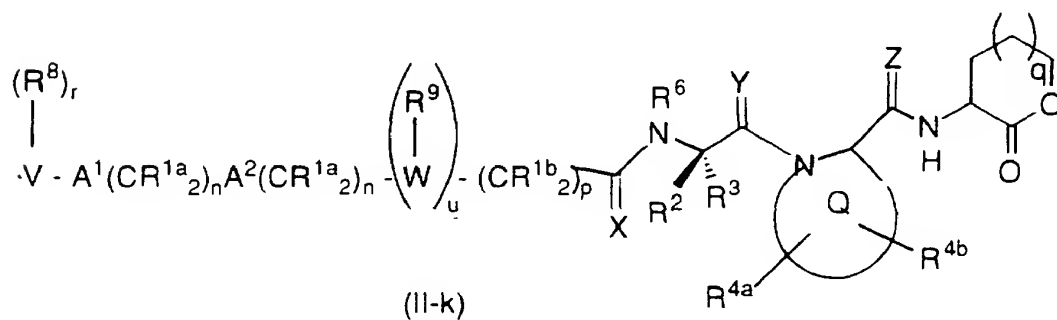
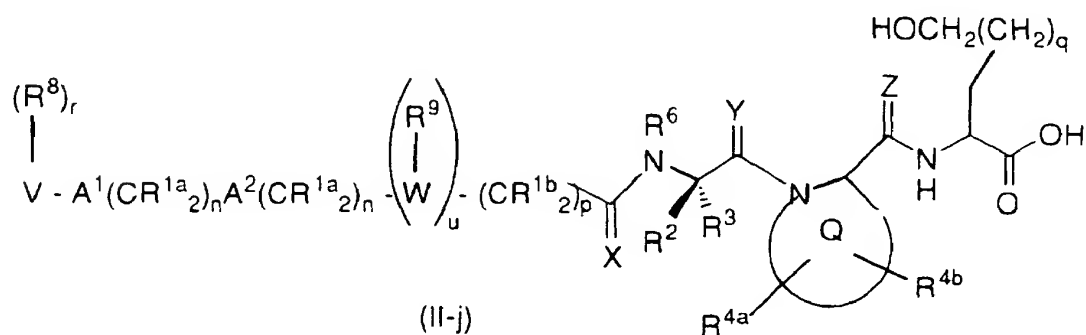
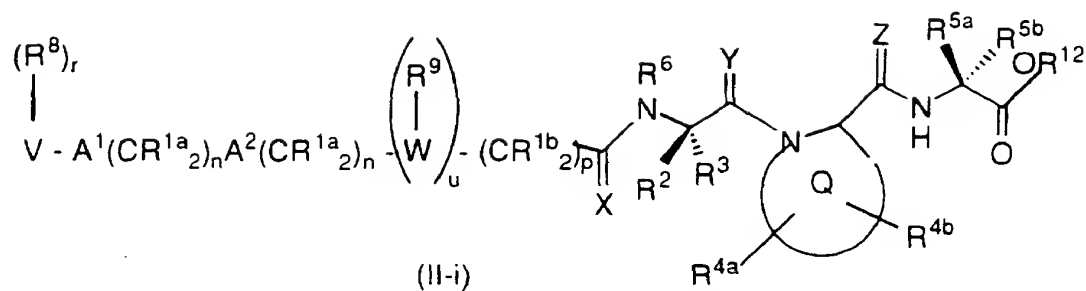
q is 0, 1 or 2;
20 s is 4 or 5;
t is 3, 4 or 5; and
u is 0 or 1;

(c) a compound represented by formula (II-h) through (II-k):

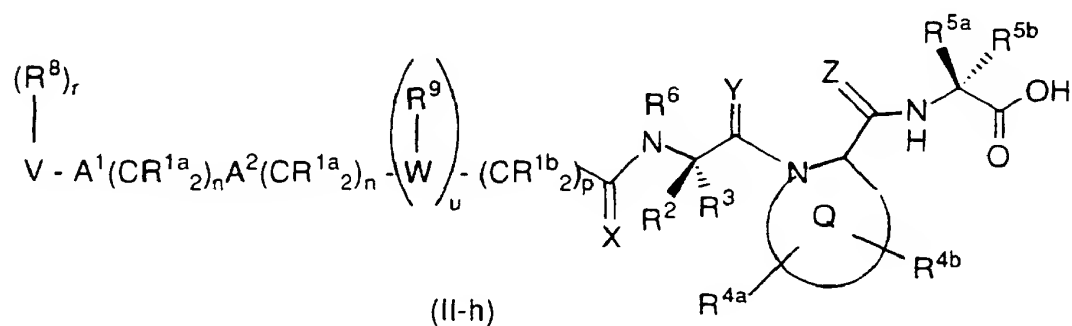
25



-44-



wherein with respect to formula (II-h):



or a pharmaceutically acceptable salt thereof,

-45-

R^{1a} , R^{1b} , R^8 , R^9 , R^{10} , R^{11} , A^1 , A^2 , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

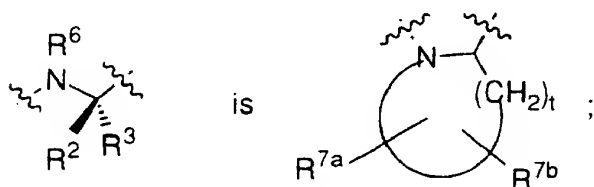
5 R^2 and R^3 are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - 10 ii) methionine sulfone, and
- c) substituted or unsubstituted C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_3 - C_{10} cycloalkyl, aryl or heterocyclyl group,

wherein the substituent is selected from F, Cl, Br, $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$,
 15 CN , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl, and
- d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and
 20 C_3 - C_{10} cycloalkyl; or

R^2 and R^3 are combined to form $-(CH_2)_s-$; or

25 R^2 or R^3 are combined with R^6 to form a ring such that



R^{4a} , R^{4b} , R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,

-46-

- b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- 5 c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;
- 10

R^{5a} and R^{5b} are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
- 15 i) methionine sulfoxide, or
- ii) methionine sulfone,
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
- 20 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or
- 25

R^{5a} and R^{5b} are combined to form - (CH₂)_s - wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)-;

30

R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

-47-

Q is a substituted or unsubstituted nitrogen-containing C4-C9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C5-C7 saturated ring or a heterocycle;

5 X, Y and Z are independently H₂ or O;

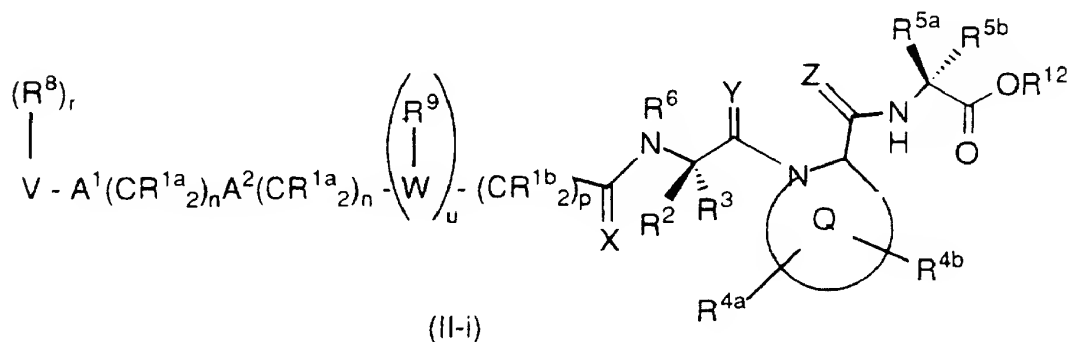
s is 4 or 5;

t is 3, 4 or 5; and

u is 0 or 1;

10

with respect to formula (II-i):



15 or a pharmaceutically acceptable salt thereof,
wherein:

R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p and r are as
previously defined with respect to formula (II-a);

20

R² and R³ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

25

-48-

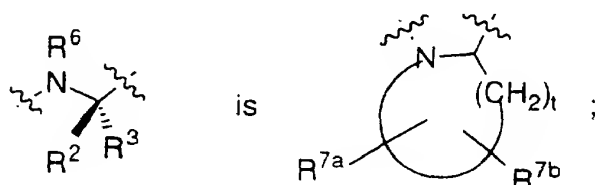
wherein the substituent is selected from F, Cl, Br,
 $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$,
 CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$,
 N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl,
 and

5

- d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

10 R^2 and R^3 are combined to form $-(CH_2)_s-$; or

R^2 or R^3 are combined with R^6 to form a ring such that



15

R^{4a} , R^{4b} , R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
 b) C_1 - C_6 alkyl unsubstituted or substituted by alkenyl, $R^{10}O-$,
 $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N_3 , $(R^{10})_2N-C(NR^{10})-$,
 20 $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
 c) aryl, heterocycle, cycloalkyl, alkenyl,
 $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 ,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
 $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}-$, and
 25 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C_3 - C_{10} cycloalkyl;

R^{5a} and R^{5b} are independently selected from:

- 30 a) a side chain of a naturally occurring amino acid,

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- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
- i) methionine sulfoxide, or
 - ii) methionine sulfone,
- 5 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group, wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
- 10 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or
- 15 R^{5a} and R^{5b} are combined to form -(CH₂)_s- wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)-

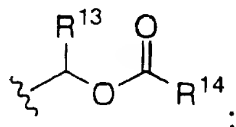
R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

20

R¹² is

- a) substituted or unsubstituted C₁-C₈ alkyl or substituted or unsubstituted C₅-C₈ cycloalkyl, wherein the substituent on the alkyl or cycloalkyl is selected from:
- 1) aryl,
 - 2) heterocycle,
 - 3) -N(R¹¹)₂,
 - 4) -OR¹⁰, or
- 25 b)

30



R¹³ is independently selected from hydrogen and C₁-C₆ alkyl;

-50-

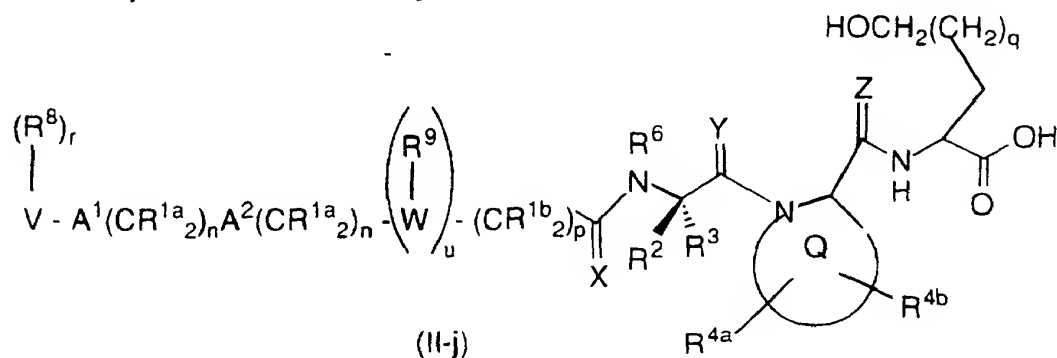
R^{14} is independently selected from C_1 - C_6 alkyl;

Q is a substituted or unsubstituted nitrogen-containing C_4 - C_9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C_5 - C_7 saturated ring or a heterocycle;

X, Y and Z are independently H_2 or O;

s is 4 or 5;
 10 t is 3, 4 or 5; and
 u is 0 or 1;

with respect to formula (II-j):



15

or a pharmaceutically acceptable salt thereof,

R^{1a} , R^{1b} , R^8 , R^9 , R^{10} , R^{11} , A^1 , A^2 , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

20

R^2 and R^3 are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and

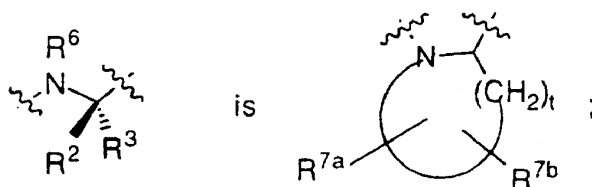
25

-51-

- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
 wherein the substituent is selected from F, Cl, Br,
 N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,
 5 CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-,
 N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 and
- d) C₁-C₆ alkyl substituted with an unsubstituted or
 substituted group selected from aryl, heterocycle and
 10 C₃-C₁₀ cycloalkyl; or

R² and R³ are combined to form - (CH₂)_s - ; or

R² or R³ are combined with R⁶ to form a ring such that
 15



R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
 20 b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-,
 R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-,
 R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 c) aryl, heterocycle, cycloalkyl, alkenyl,
 R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂,
 25 (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃,
 -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or
 substituted group selected from aryl, heterocyclyl and
 30 C₃-C₁₀ cycloalkyl;

R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

-52-

Q is a substituted or unsubstituted nitrogen-containing C4-C9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C5-C7 saturated ring or a heterocycle;

5

X, Y and Z are independently H₂ or O;

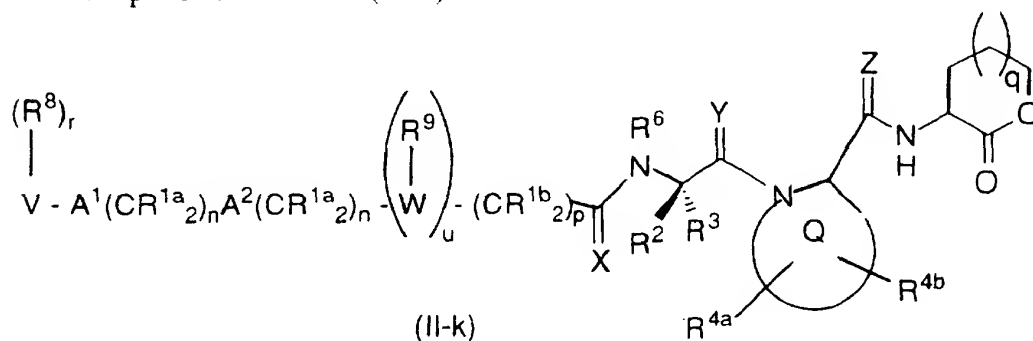
q is 0, 1 or 2;

s is 4 or 5;

10 t is 3, 4 or 5; and

u is 0 or 1;

with respect to formula (II-k):



15

or a pharmaceutically acceptable salt thereof,

R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p, and r are as defined above with respect to formula (II-a);

20

R² and R³ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

25

-53-

wherein the substituent is selected from F, Cl, Br, $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl,

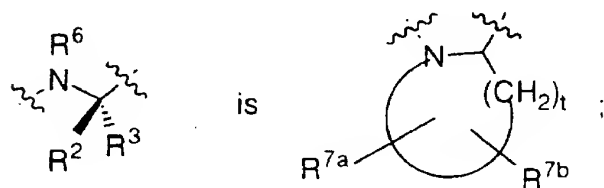
5

and

- d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

10 R^2 and R^3 are combined to form $-(CH_2)_s-$; or

R^2 or R^3 are combined with R^6 to form a ring such that



15

R^{4a} , R^{4b} , R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
- b) C_1 - C_6 alkyl unsubstituted or substituted by alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , N_3 , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- 20 c) aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , NO_2 , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}-$, and
- 25 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C_3 - C_{10} cycloalkyl;

30

R^6 is independently selected from hydrogen or C_1 - C_6 alkyl;

-54-

Q is a substituted or unsubstituted nitrogen-containing C4-C9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C5-C7 saturated ring or a heterocycle;

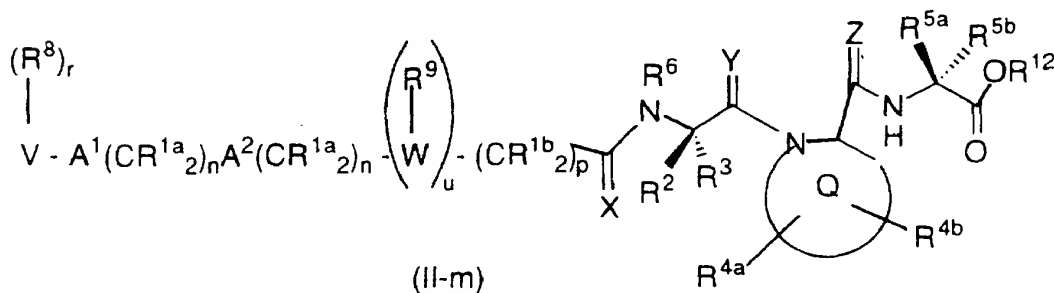
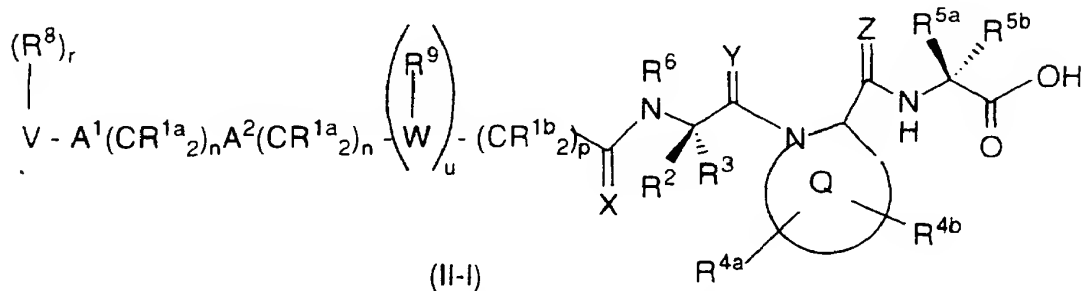
5 X, Y and Z are independently H₂ or O;

q is 0, 1 or 2;
 s is 4 or 5;
 t is 3, 4 or 5; and
 10 u is 0 or 1;

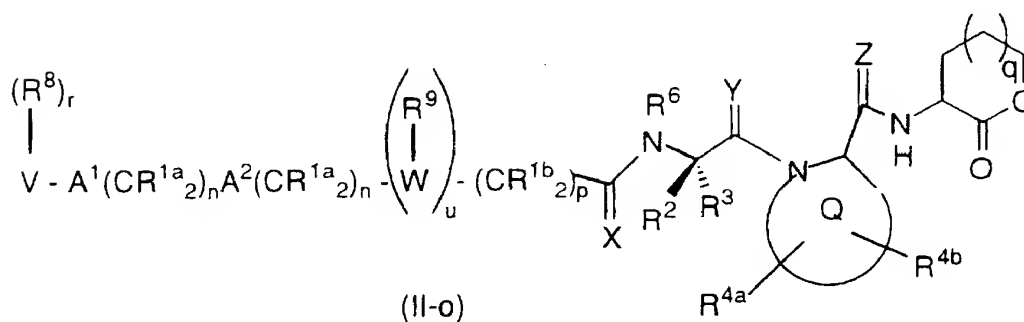
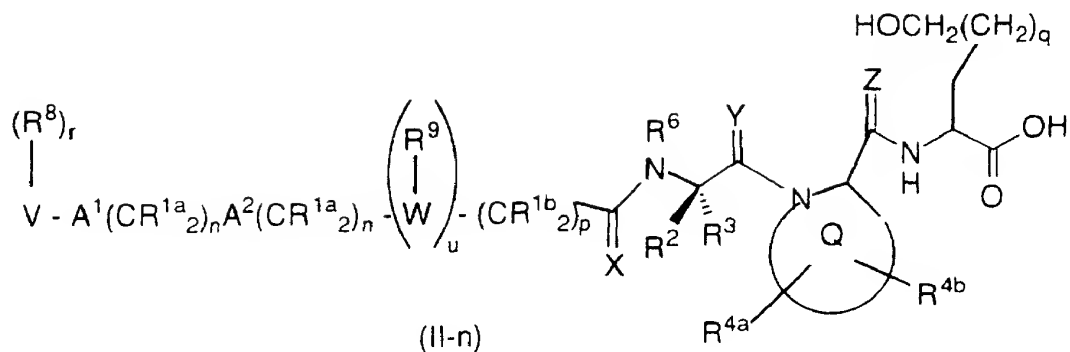
and

(d) a compound represented by formula (II-l) through (II-o):

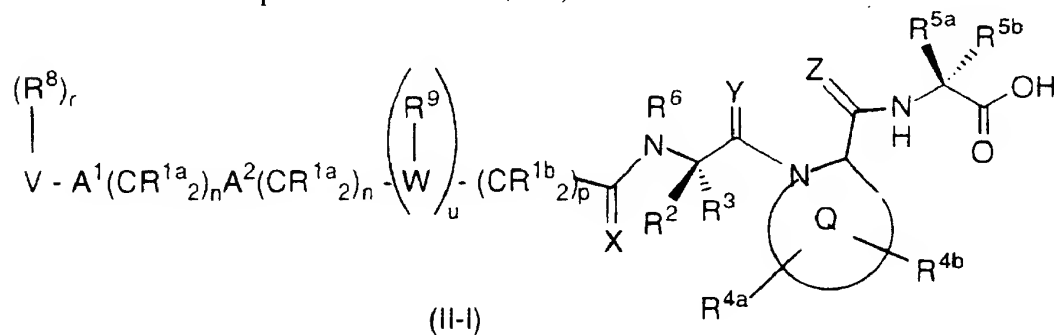
15



-55-



wherein with respect to formula (II-l):



5

or a pharmaceutically acceptable salt thereof:

R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p and r are as defined above with respect to formula (II-a);

10

R² and R³ are independently selected from:

a) a side chain of a naturally occurring amino acid.

-56-

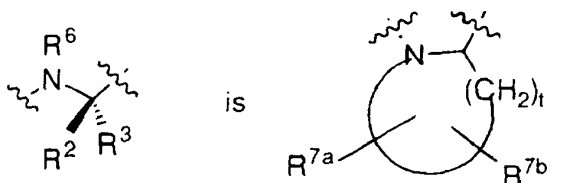
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
- methionine sulfoxide, or
 - methionine sulfone, and
- 5 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group, wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl.
- 10 and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃- C₁₀ cycloalkyl; or

15

R² and R³ are combined to form - (CH₂)_s - ; or

R² or R³ are combined with R⁶ to form a ring such that

20



R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- hydrogen,
 - 25 C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 - c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
- 30

-57-

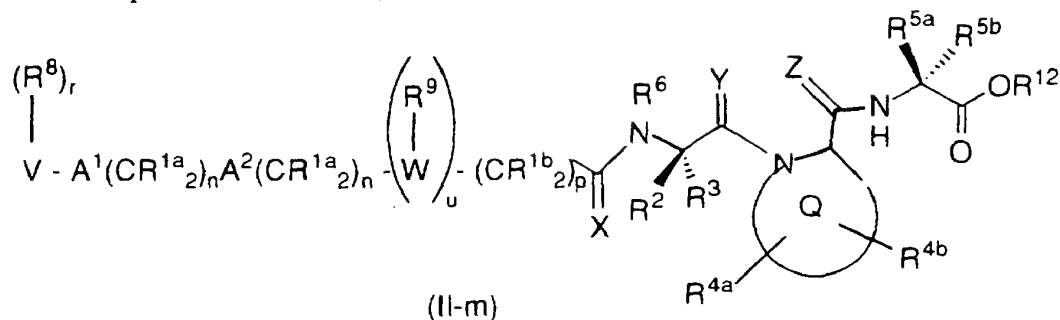
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;
- 5 R^{5a} and R^{5b} are independently selected from:
- a side chain of a naturally occurring amino acid,
 - an oxidized form of a side chain of a naturally occurring amino acid which is:
 - methionine sulfoxide, or
 - methionine sulfone,
 - substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,

wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 - C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or
- 20 R^{5a} and R^{5b} are combined to form - (CH₂)_s - wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)- ;
- 25 R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;
- Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;
- 30 X, Y and Z are independently H₂ or O;
- s is 4 or 5;
- t is 3, 4 or 5; and

-58-

u is 0 or 1;

with respect to formula (II-m):



5 or a pharmaceutically acceptable salt thereof,

R^{1a} , R^{1b} , R^8 , R^9 , R^{10} , R^{11} , A^1 , A^2 , V , W , m , n , p and r are as defined above with respect to formula (II-a);

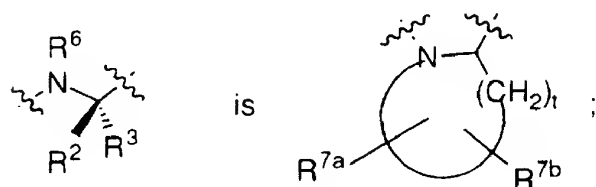
10 R^2 and R^3 are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- 15 c) substituted or unsubstituted C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_3 - C_{10} cycloalkyl, aryl or heterocyclyl group, wherein the substituent is selected from F, Cl, Br, $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl, and
- 20 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or
- 25

R^2 and R^3 are combined to form $-(CH_2)_s-$; or

-59-

R² or R³ are combined with R⁶ to form a ring such that



- 5 R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:
- a) hydrogen,
 - b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 - 10 c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
 - d) C₁-C₆ alkyl substituted with an unsubstituted or
 - 15 substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

R^{5a} and R^{5b} are independently selected from:

- a) a side chain of a naturally occurring amino acid,
 - 20 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone,
 - c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl,
 - 25 C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
- wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,

-60-

- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

5 R^{5a} and R^{5b} are combined to form - (CH₂)_s - wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)- ;

R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

10

R¹² is

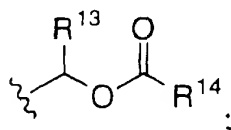
- a) substituted or unsubstituted C₁-C₈ alkyl or substituted or unsubstituted C₅-C₈ cycloalkyl, wherein the substituent on the alkyl or cycloalkyl is selected from:

15

- 1) aryl,
- 2) heterocycle,
- 3) -N(R¹¹)₂,
- 4) -OR¹⁰, or

b)

20



R¹³ is independently selected from hydrogen and C₁-C₆ alkyl;

R¹⁴ is independently selected from C₁-C₆ alkyl;

25

Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;

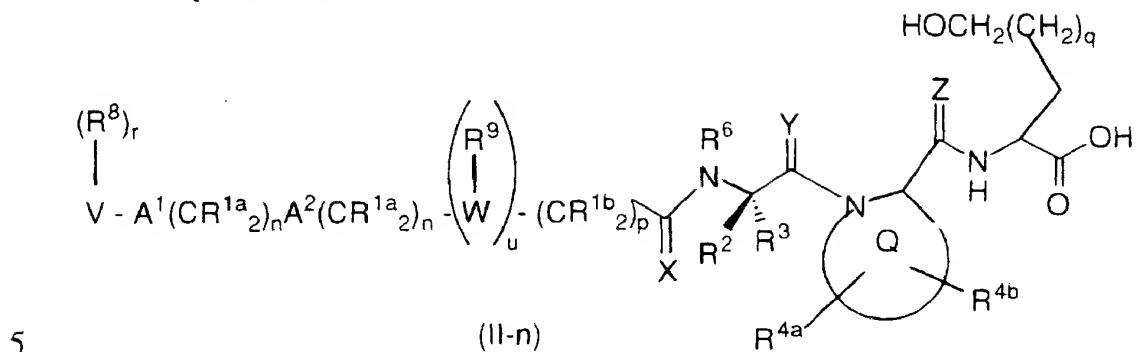
30 X, Y and Z are independently H₂ or O;

s is 4 or 5;

-61-

t is 3, 4 or 5; and
u is 0 or 1;

with respect to formula (II-n):



or a pharmaceutically acceptable salt thereof:

10 R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p and r are as defined above with respect to formula (II-a);

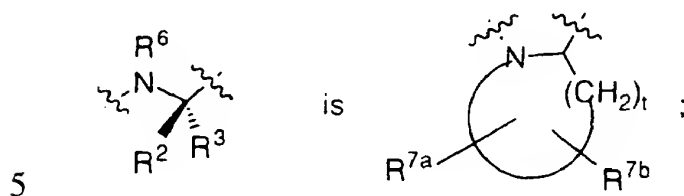
R^2 and R^3 are independently selected from:

- 15 a) a side chain of a naturally occurring amino acid,
b) an oxidized form of a side chain of a naturally occurring amino acid which is:
i) methionine sulfoxide, or
ii) methionine sulfone, and
c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
20 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl, and
25 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

-62-

R² and R³ are combined to form $-(CH_2)_s-$; or

R² or R³ are combined with R⁶ to form a ring such that



R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- 10
- a) hydrogen,
 - b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 - 15 c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
 - d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

20 R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;

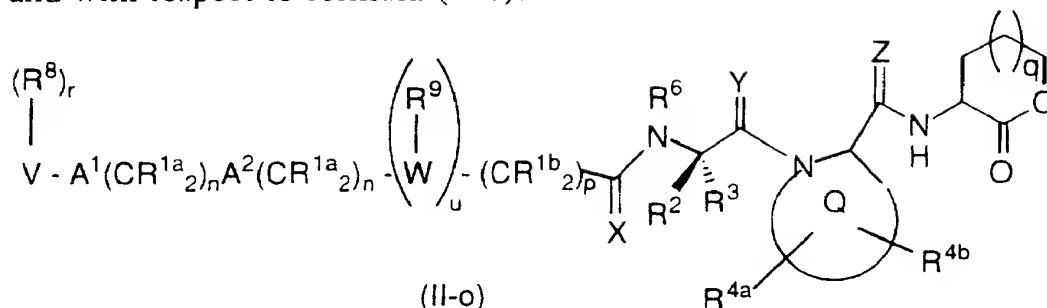
25

X, Y and Z are independently H₂ or O;

- q is 0, 1 or 2;
 s is 4 or 5;
 30 t is 3, 4 or 5; and
 u is 0 or 1;

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and with respect to formula (II-o):



5 or a pharmaceutically acceptable salt thereof:

R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p and r are as defined above with respect to formula (II-a);

10 R² and R³ are independently selected from:

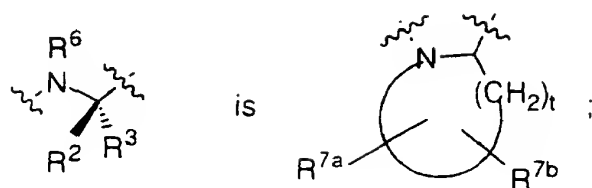
- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

R² and R³ are combined to form - (CH₂)_s - ; or

R² or R³ are combined with R⁶ to form a ring such that

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R^{4a} , R^{4b} , R^{7a} and R^{7b} are independently selected from:

- 5 a) hydrogen,
- b) C_1 - C_6 alkyl unsubstituted or substituted by alkenyl, $R^{10}O$ -, $R^{11}S(O)_m$ -, $R^{10}C(O)NR^{10}$ -, CN , N_3 , $(R^{10})_2N-C(NR^{10})$ -, $R^{10}C(O)$ -, $R^{10}OC(O)$ -, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$ -,
- c) aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$ -, $R^{11}S(O)_m$ -, $R^{10}C(O)NR^{10}$ -, CN , NO_2 , $(R^{10})_2N-C(NR^{10})$ -, $R^{10}C(O)$ -, $R^{10}OC(O)$ -, N_3 , $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}$ -, and
- 10 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and
- 15 C_3 - C_{10} cycloalkyl;

R^6 is independently selected from hydrogen or C_1 - C_6 alkyl;

20 Q is a substituted or unsubstituted nitrogen-containing C_4 - C_9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C_5 - C_7 saturated ring or a heterocycle;

X , Y and Z are independently H_2 or O ;

- 25 q is 0, 1 or 2;
- s is 4 or 5;
- t is 3, 4 or 5; and
- u is 0 or 1.

30 Specific compounds which antagonize Raf include the following:

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- 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;
- 5 4-[4-fluorophenyl)-3-pyridin-yl-1H-imidazol-2-yl]-1-acetyl-piperidine;
- 3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;
- 10 3-[4-fluorophenyl)-3-pyridin-yl-1H-imidazol-2-yl]-1-acetyl-piperidine;
and
- 4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester.
- 15 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;
- 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;
- 20 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-benzyl-piperidine;
- 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-ethyl-piperidine;
- 25 4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;
- 4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;
- 30 2-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-butyl)-isoindole-1,3-dione;
- 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-isoindole-1,3-dione;

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2-(6-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-hexyl)-isoindole-1,3-dione;

- 5 4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-benzyl-piperidine;

- 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-2,3-dihydro-isoindol-1-one ditrifluoroacetic
10 acid salt;

4-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-ethyl)-pyridine;

- 15 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-1,1-dioxobenzo[d]isothiazol-3-one;

2-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-butyl)-1,1-dioxobenzo[d]isothiazol-3-one;

- 20 2-amino-1-{5-[4-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-ethanone dihydrochloride;

- 25 4-[5-(3-hydroxyphenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;

- 30 3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine:

3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

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- 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1,4-dimethyl-piperidine;
- 5 4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;
- 4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;
- 10 4-{5-(3,4-dichlorophenyl)-2-[1-(2-phenylethyl)-piperidin-4-yl]-1H-imidazol-4-yl}-pyridine;
- 4-{5-(3,4-dichlorophenyl)-2-[1-(3-phenylpropyl)-piperidin-4-yl]-1H-imidazol-4-yl}-pyridine;
- 15 2-(6-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-hexyl)-1,1-dioxobenzo[d]isothiazol-3-one;
- 20 2-(3-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-propyl)-1,1-dioxobenzo[d]isothiazol-3-one;
- 4-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl-methyl}-imidazol-1-yl-methyl)-benzonitrile;
- 25 4-[2-[1-(4-benzyloxybenzyl)-piperidin-4-yl-5-(3,4-dichlorophenyl)-1H-imidazol-4-yl]-pyridine;
- 2-(3-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-propyl)-isoindole-1,3-dione;
- 30 4-[4-(4-fluorophenyl)-5-(4-pyridyl)imidazol-2-yl]benzamidoxime;
- 4-(1-naphthyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;

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- 4-(1-naphthyl)-2-(4-methylthiophenyl)-5-(4-pyridyl)imidazole;
- 4-(2-naphthyl)-2-(4-methylthiophenyl)-5-(4-pyridyl)imidazole;
- 5 4-(2-naphthyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(3-thiophenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(2-thiophenyl)-5-(4-pyridyl)imidazole;
- 10 4-(4-fluorophenyl)-2-(3-methylthiophenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(3-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;
- 15 4-(4-fluorophenyl)-2-(3-methylsulfonylphenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(2-methylthiophenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(2-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;
- 20 4-(4-fluorophenyl)-2-(2-methylsulfonylphenyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(4-methoxyphenyl)-5-(4-pyridyl)imidazole;
- 25 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1-methyl-5-(4-pyridyl)
imidazole;
- 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1-(N-morpholinopropyl)-5-(4-pyridyl)imidazole;
- 30 4-(4-fluorophenyl)-2-(4-methylthiophenyl)-1-(N-morpholinopropyl)-5-(4-pyridyl)imidazole;
- 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-1-(N-morpholino-

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propyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-1-(methylthio-1-propyl)-2-([4-N-morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole;

5

4-(4-fluorophenyl)-1-(methylsulfinyl-1-propyl)-2-([4-N-morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole; and

4-(4-fluorophenyl)-1-(methylsulfonyl-1-propyl)-2-([4-N-morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole.

10

Examples of compounds which antagonize or inhibit farnesyl protein transferase include the following:

15 2(S)-Butyl-1-(2,3-diaminoprop-1-yl)-1-(1-naphthoyl)piperazine;

1-(3-Amino-2-(2-naphthylmethylamino)prop-1-yl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

20 2(S)-Butyl-1-{5-[1-(2-naphthylmethyl)]-4,5-dihydroimidazol}methyl-4-(1-naphthoyl)piperazine;

1-[5-(1-Benzylimidazol)methyl]-2(S)-butyl-4-(1-naphthoyl)piperazine;

25 1-{5-[1-(4-nitrobenzyl)]imidazolymethyl}-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-(3-Acetamidomethylthio-2(R)-aminoprop-1-yl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

30

2(S)-Butyl-1-[2-(1-imidazolyl)ethyl]sulfonyl-4-(1-naphthoyl)piperazine;

2(R)-Butyl-1-imidazolyl-4-methyl-4-(1-naphthoyl)piperazine;

35 2(S)-Butyl-4-(1-naphthoyl)-1-(3-pyridylmethyl)piperazine;

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1-2(S)-butyl-(2(R)-(4-nitrobenzyl)amino-3-hydroxypropyl)-4-(1-naphthoyl)piperazine;

5 1-(2(R)-Amino-3-hydroxyheptadecyl)-2(S)-butyl-4-(1-naphthoyl)-piperazine;

2(S)-Benzyl-1-imidazolyl-4-methyl-4-(1-naphthoyl)piperazine;

10 1-(2(R)-Amino-3-(3-benzylthio)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-(2(R)-Amino-3-[3-(4-nitrobenzylthio)propyl])-2(S)-butyl-4-(1-naphthoyl)piperazine;

15

2(S)-Butyl-1-[(4-imidazolyl)ethyl]-4-(1-naphthoyl)piperazine;

2(S)-Butyl-1-[(4-imidazolyl)methyl]-4-(1-naphthoyl)piperazine;

20 2(S)-Butyl-1-[(1-naphth-2-ylmethyl)-1H-imidazol-5-yl]acetyl]-4-(1-naphthoyl)piperazine;

2(S)-Butyl-1-[(1-naphth-2-ylmethyl)-1H-imidazol-5-yl]ethyl]-4-(1-naphthoyl)piperazine;

25

1-(2(R)-Amino-3-hydroxypropyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-(2(R)-Amino-4-hydroxybutyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

30 1-(2-Amino-3-(2-benzyloxyphenyl)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-(2-Amino-3-(2-hydroxyphenyl)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

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1-[3-(4-imidazolyl)propyl]-2(S)-butyl-4-(1-naphthoyl)-piperazine;

5 2(S)-*n*-Butyl-4-(2,3-dimethylphenyl)-1-(4-imidazolylmethyl)-
piperazin-5-one;

2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-
dimethylphenyl)piperazin-5-one;

10 1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-
2(S)-(2-methoxyethyl)piperazin-5-one;

2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(1-naphthylmethyl)imidazol-5-
ylmethyl]-piperazine;

15 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(2-naphthylmethyl)imidazol-5-
ylmethyl]-piperazine;

20 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine;

2(S)-*n*-Butyl-1-[1-(4-methoxybenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine;

25 2(S)-*n*-Butyl-1-[1-(3-methyl-2-butenyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine;

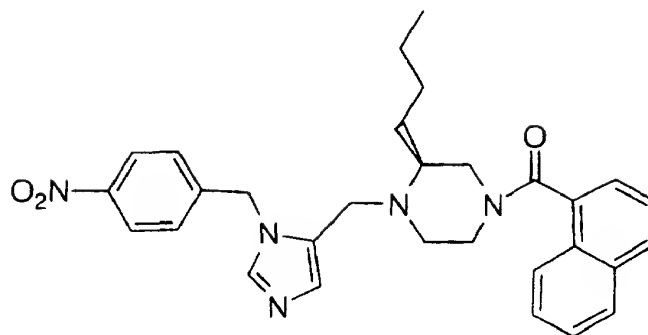
2(S)-*n*-Butyl-1-[1-(4-fluorobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine;

30 2(S)-*n*-Butyl-1-[1-(4-chlorobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine;

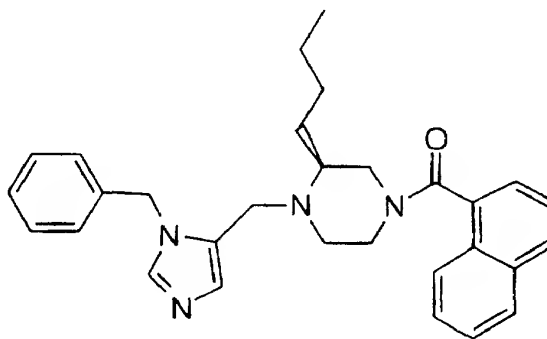
-72-

- 1-[1-(4-Bromobenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-naphthoyl)piperazine;
- 5 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethylbenzyl)imidazol-5-ylmethyl]-piperazine;
- 2(S)-*n*-Butyl-1-[1-(4-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine;
- 10 2(S)-*n*-Butyl-1-[1-(3-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine;
- 1-[1-(4-Phenylbenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-naphthoyl)-piperazine;
- 15 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(2-phenylethyl)imidazol-5-ylmethyl]-piperazine;
- 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethoxy)imidazol-5-ylmethyl]piperazine;
- 20 1-([1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetyl)-2(S)-*n*-butyl-4-(1-naphthoyl)piperazine;
- 25 1-[5-[1-(4-nitrobenzyl)]imidazolylmethyl]-2(S)-butyl-4-(1-naphthoyl)piperazine

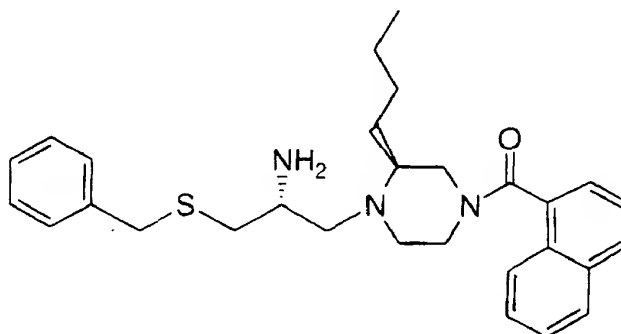
-73-



1-[5-(1-Benzylimidazol)methyl]-2(S)-butyl-4-(1-naphthoyl)piperazine

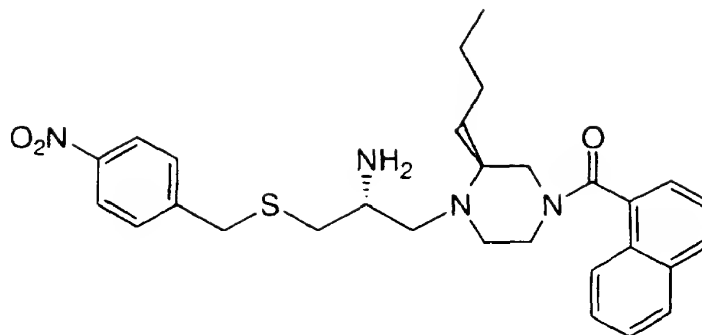


1-(2(R)-Amino-3-(3-benzylthio)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine

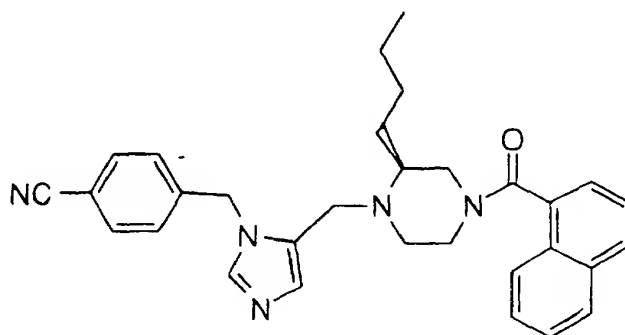


1-(2(R)-Amino-3-[3-(4-nitrobenzylthio)propyl])-2(S)-butyl-4-(1-naphthoyl)piperazine

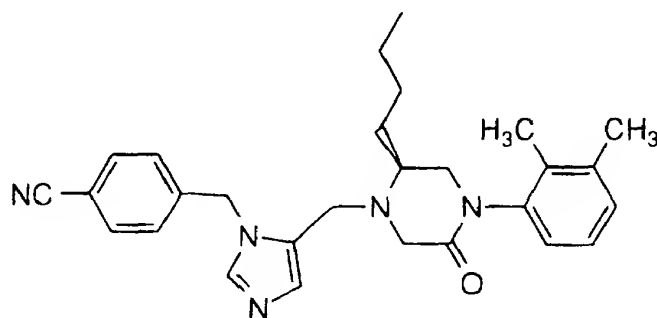
-74-



2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine

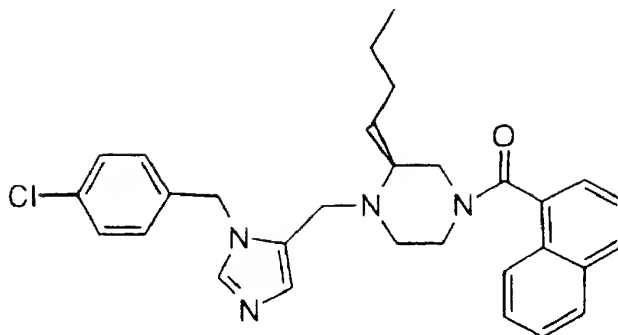


5 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)piperazin-5-one

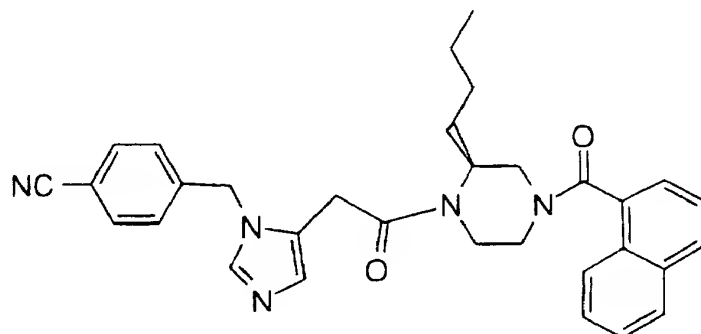


2(S)-*n*-Butyl-1-[1-(4-chlorobenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine

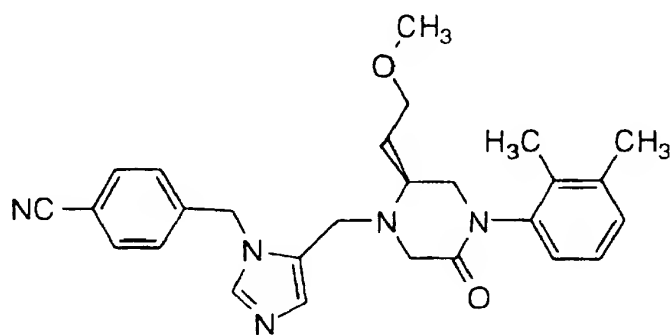
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1-([1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetyl)-2(S)-n-butyl-4-(1-naphthoyl)piperazine

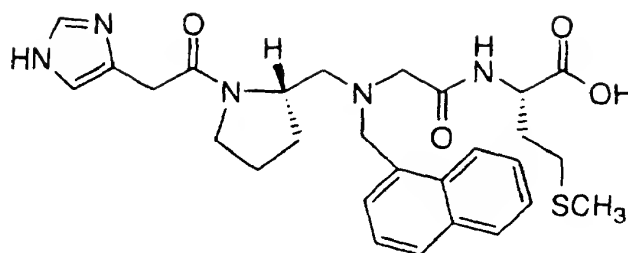


5 1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-2(S)-(2-methoxyethyl)piperazin-5-one



10 N-[1-(4-Imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycylmethionine

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N-[1-(4-Imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

- 5 N-[1-(2(S),3-Diaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(2(S),3-Diaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

10

N-[1-(3-Aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 15 N-[1-(3-Aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(2(S)-Amino-3-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 20 N-[1-(2(S)-Amino-3-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

- 25 N-[1-(3-Amino-2(S)-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(3-Amino-2(S)-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(L-Glutaminyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 5 N-[1-(L-Glutaminyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(L-Histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ;

10

N-[1-(L-Histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

- 15 N-[1-(D-Histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(D-Histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

- 20 N-[1-(L-Pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(L-Pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester ;

25

2(S)-[1-(2(S)-Pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

- 30 2(S)-[1-(2(S)-Pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(2(S)-Pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine isopropyl ester;

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2(S)-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

5 2(S)-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(2(S)-Pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine sulfone;

10 2(S)-[1-(2(S)-Pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine sulfone methyl ester;

2(S)-[1-(Pyrid-3-ylcarboxy)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

15 2(S)-[1-(Pyrid-3-ylcarboxy)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

20 2(R)-{2-[1-(Naphth-2-yl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenylpropionyl-methionine;

2(R)-{2-[1-(Naphth-2-yl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenylpropionyl-methionine methyl ester;

25 2(S)-[1-(Pyrid-3-ylmethyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

2(S)-[1-(Pyrid-3-ylmethyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

30 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone isopropyl ester;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

10 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ;

15 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone methyl ester ;

20 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone;

N-[1-(Sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

25 N-[1-(Sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(N,N-Dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester ;

30 N-[1-(N,N-Dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine methyl ester;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(Glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

10 N-[1-(Glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

15 N-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

20 N-[1-(2-Acetylamino-3(S)-benzyloxycarbonylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

25 N-[1-(2-Acetylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(2-Amino-3(S)-acetylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

30 2(S)-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

-81-

2(R)-{2-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester ;

5 2(R)-{2-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

2(R)-{2-[1-(4-Nitrobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

10 2(R)-{2-[1-(4-Nitrobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

2(R)-{2-[1-(4-Methoxybenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

15 2(R)-{2-[1-(4-Methoxybenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

20 2(R)-{2-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

2(R)-{2-[1-(4-Cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

25 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine;

30 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

-82-

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-
(β -acetylamino)alanine;

5 N-[1-(Seryl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-
methionine methyl ester;

N-[1-(D-Alanyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-
methionine methyl ester;

10 N-[1-(1H-imidazol-4-carbonyl)pyrrolidin-2(S)-ylmethyl]- N-(1-
naphthylmethyl)glycyl-methionine methyl ester;

15 N-[1-(Isoasparagyl) pyrrolidin-2(S)-ylmethyl]-N-(1-
naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]- N-(1-
naphthylmethyl)glycyl-methionine methyl ester;

20 N-[1-(3-Pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-
naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(2-Pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-
naphthylmethyl)glycyl-methionine methyl ester ;

25 N-[1-(4-Pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-
naphthylmethyl)glycyl-methionine methyl ester;

30 N-[1-(Seryl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-
methionine;

N-[1-(D-Alanyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-
methionine;

35 N-[1-(1H-Imidazol-4-carbonyl)pyrrolidin-2(S)-ylmethyl]- N-(1-
naphthylmethyl)glycyl-methionine ;

-83-

N-[1-(Isoasparagyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

5 N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(3-Pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

10 N-[1-(2-Pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(4-Pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

15 N-[1-(1H-Imidazol-4-ylmethyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

20 N-[1-(2-Aminoethyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(2-thienyl)alanine;

25 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(trifluoromethyl)alanine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(2(S)-amino-4-acetylamino)butyric acid ;

30 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N,N-dimethyl)glutamine;

-84-

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

5

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(4-methoxybenzyl)glycyl-methionine;

N-[1-(Glycyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

10

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

15 N-((4-Imidazolyl)methyl-(2S)-pyrrolidinylmethyl)-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(2-thienyl)alanine methyl ester;

20

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N,N-dimethyl)glutamine methyl ester ;

25 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(trifluoromethyl)alanine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(2(S)-amino-4-acetylamino)butyric acid methyl ester;

30

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine methyl ester;

-85-

N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]-N-(benzyl)glycyl-methionine methyl ester;

5 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(4-methoxybenzyl)glycyl-methionine methyl ester;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(benzyl)glycyl-methionine methyl ester;

10 N-[1-(Glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(benzyl)glycyl-methionine methyl ester;

15 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine cyclohexyl ester;

20 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine benzyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ethyl ester;

25 N-[1-(Sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

30 N-[1-(N,N-Dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine (2-pyridylmethyl) ester;

35 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine (1-glyceryl) ester;

-86-

N-[1-L-Prolylpyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

5 N-[1-(L-Prolyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(1-Morpholinoacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

10 N-[1-(1-Morpholinoacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(4-Piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

15 N-[1-(4-Piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

20 N-[1-(3-Piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(3-Piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

25 N-[1-(2-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(2-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

30 N-[1-(4-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(4-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

5 N-[1-(4-Pyridyl(N-methyl)glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(4-Pyridyl(N-methyl)glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

10 N-[1-(1H-Imidazol-4-ylpropionyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine;

N-[1-(1H-Imidazol-4-ylpropionyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

15 N-[1-(4-Pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine;

20 N-[1-(4-Pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine cyclohexyl ester;

25 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N-methyl)glutamine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N-methyl)glutamine methyl ester ;

30 N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylcarbonylamino)alanine;

-88-

N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylcarbonylamino)alanine methyl ester;

5 N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylsulfonylamino)alanine;

N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylsulfonylamino)alanine methyl ester;

10 N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -propionylamino)alanine ;

N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -propionylamino)alanine methyl ester;

15 N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -pyrrolidinon-1-ylamino)alanine;

20 N-[1-(1H-Imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -pyrrolidinon-1-ylamino)alanine methyl ester;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine;

25 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine methyl ester;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

30 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

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N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine;

5 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine methyl ester;

N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

10 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

15 N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

20 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-cyanobenzyl)glycyl-methionine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-cyanobenzyl)glycyl-methionine methyl ester ;

25 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(4-cyanobenzyl)glycyl-methionine;

30 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

-90-

N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

5 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

10 N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methylbenzyl)glycyl-methionine;

15 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methylbenzyl)glycyl-methionine methyl ester;

20 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-trifluoromethylbenzyl)glycyl-methionine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-trifluoromethylbenzyl)glycyl-methionine methyl ester;

25 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylsulfonyl)glycyl-methionine;

N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylsulfonyl)glycyl-methionine methyl ester;

30 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine 4-N-methylpiperidinyl ester;

-91-

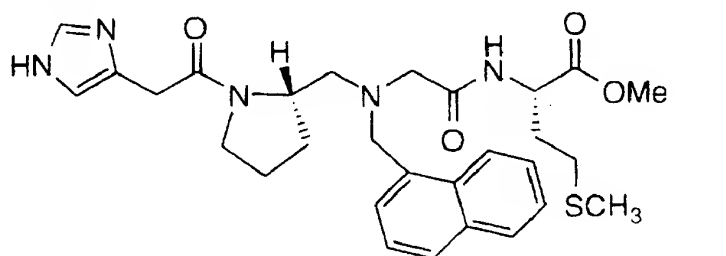
N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine tert-butyl ester;

5 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine 3-pentyl ester;

N-[1-(4-Pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

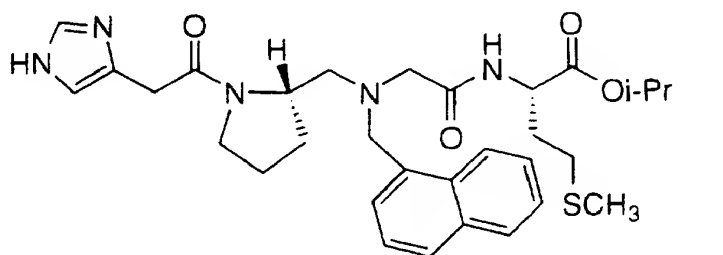
10 N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(4-Imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester



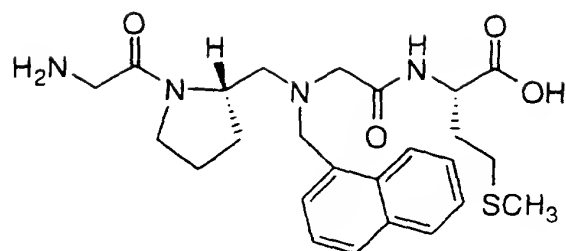
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N-[1-(4-Imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester

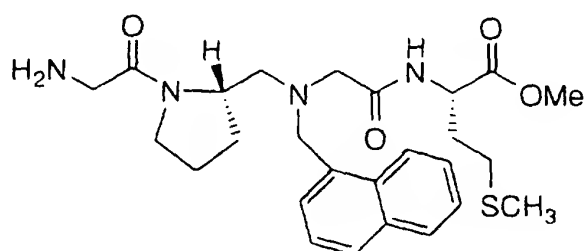


20 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine

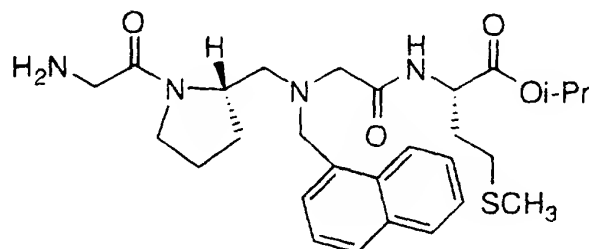
-92-



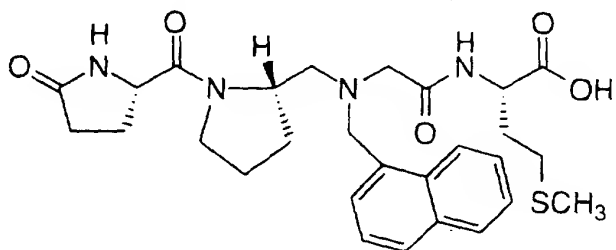
N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester



- 5 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester

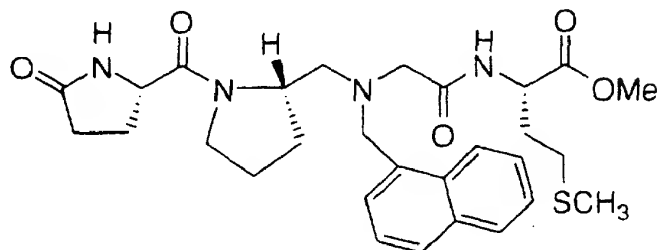


N-[1-(L-Pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine

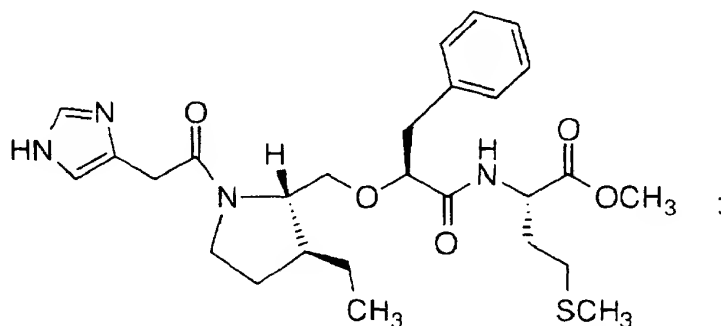


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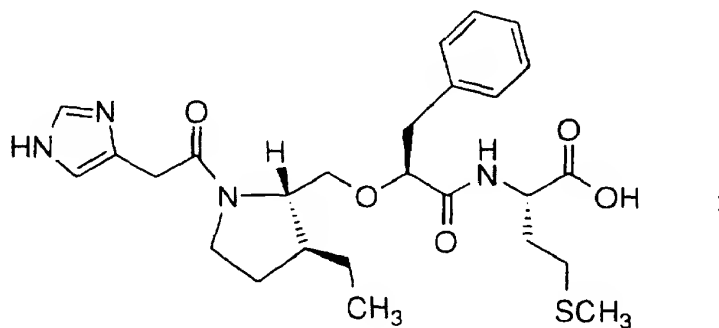
N-[1-(L-Pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester



2(S)-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester

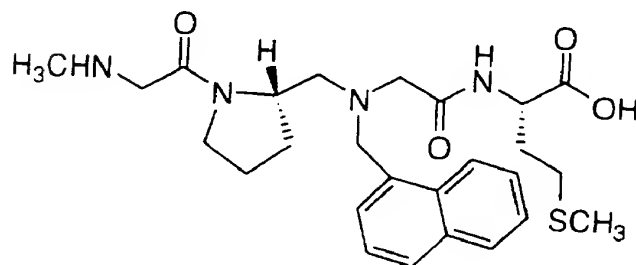


2(S)-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine

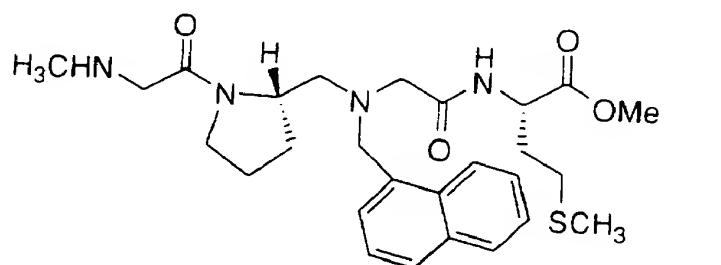


10 N-[1-(Sarcosyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine

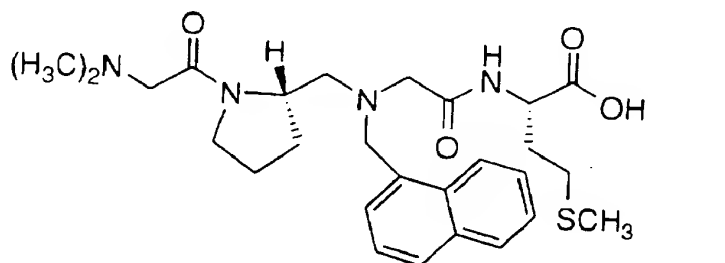
-94-



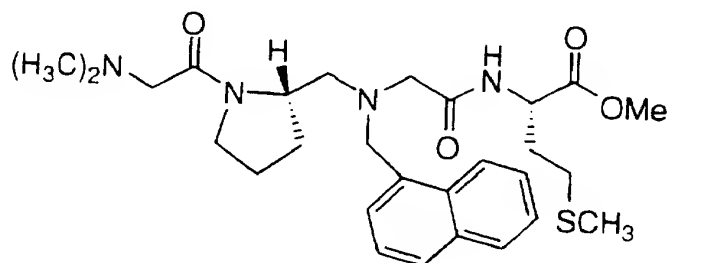
N-[1-(Sarcosyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester



- 5 N-[1-(N,N-Dimethylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine



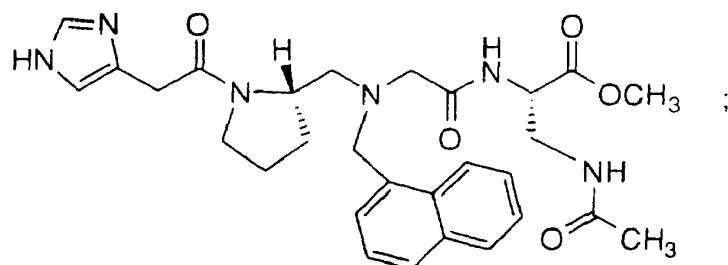
N-[1-(N,N-Dimethylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester



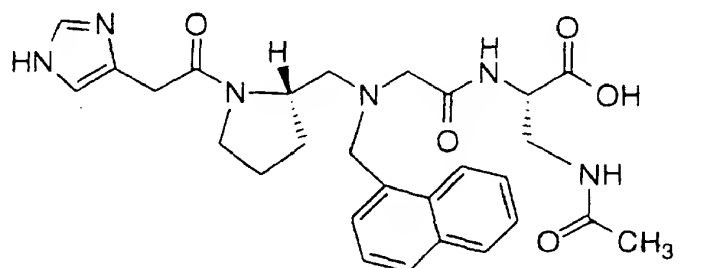
10

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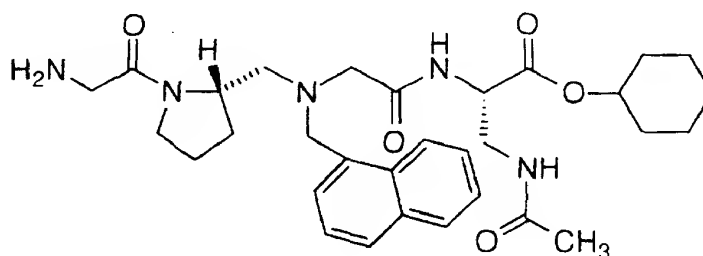
N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester



5 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine

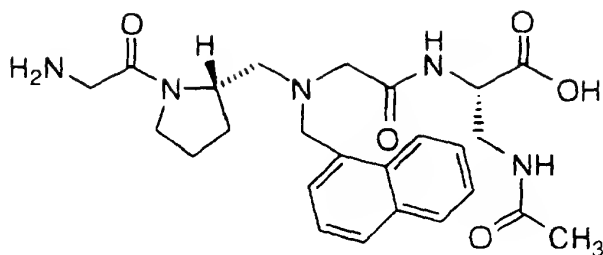


N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine cyclohexyl ester

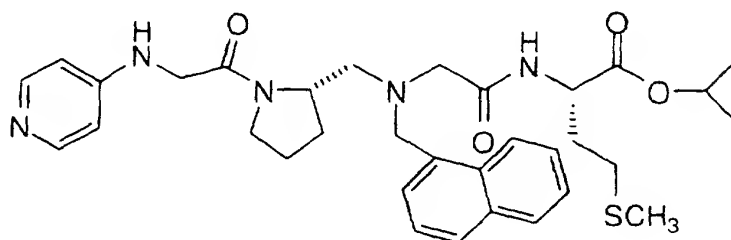


10 N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine

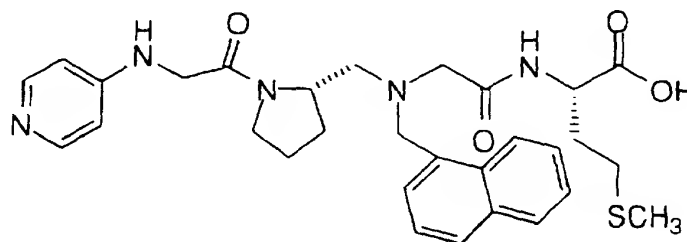
-96-



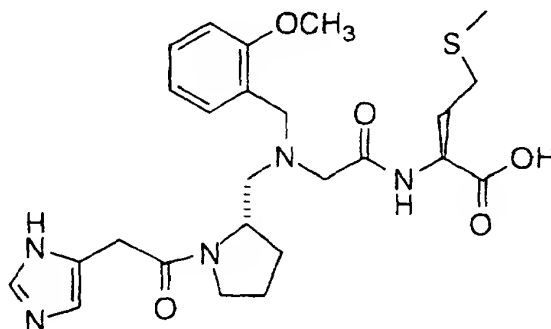
N-[1-(4-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester



- 5 N-[1-(4-Pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine

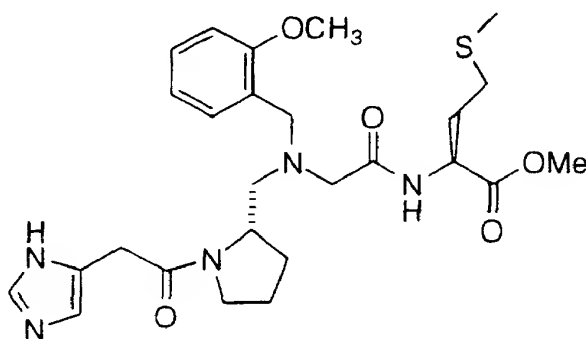


N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(2-methoxybenzyl)glycyl-methionine

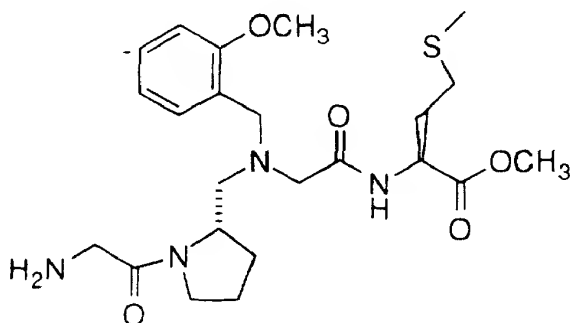


-97-

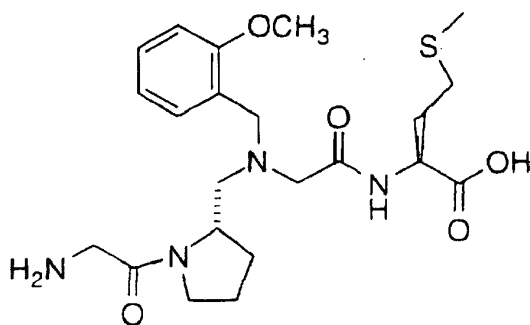
N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester



5 N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester

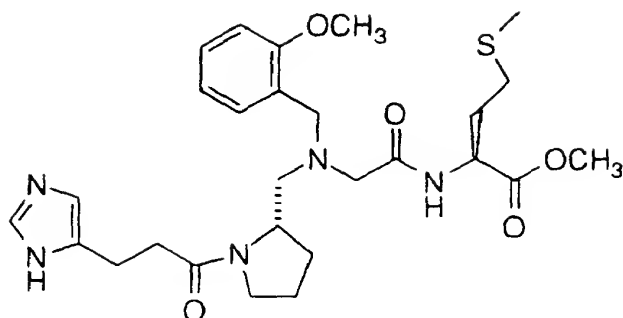


N-[1-(Glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine

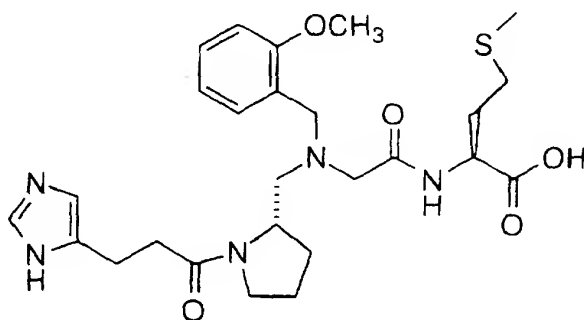


10 N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester

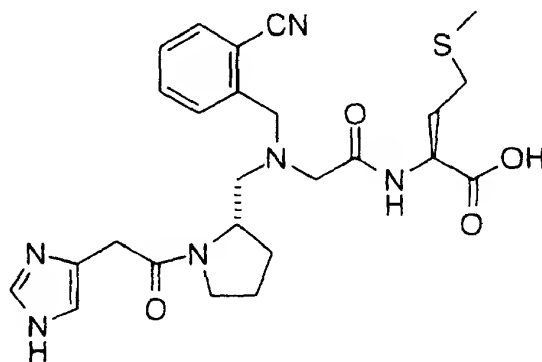
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N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine

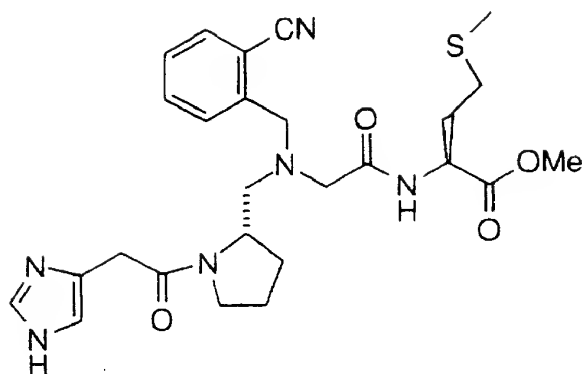


- 5 N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine

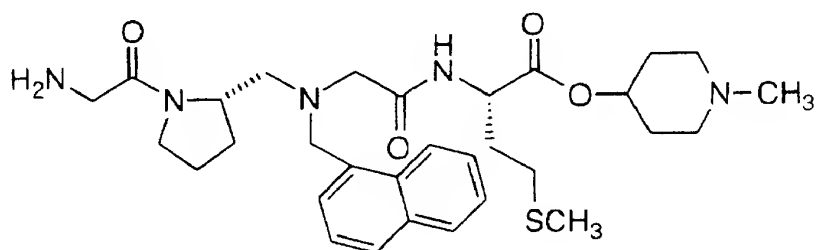


N-[1-(1H-Imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester

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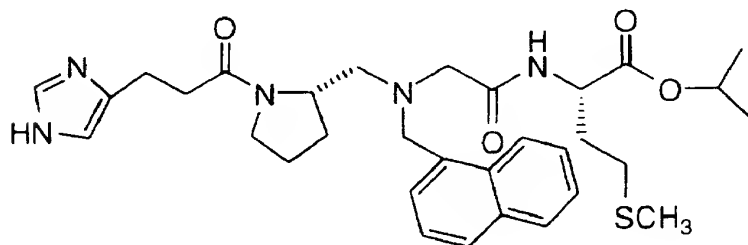


N-[1-(Glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine 4-N-methylpiperidiny ester



5

N-[1-(1H-Imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine isopropyl ester



10

N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine methyl ester;

15 N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine;

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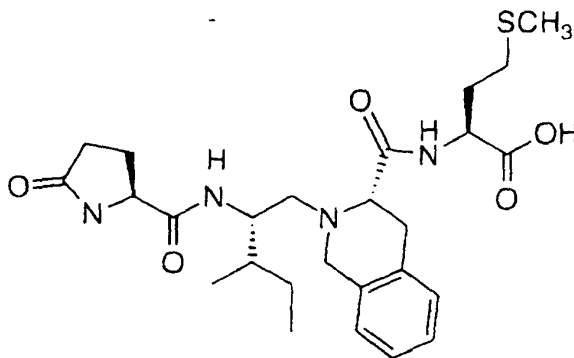
N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-
prolyl-methionine methyl ester;

5 N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-
prolyl-methionine;

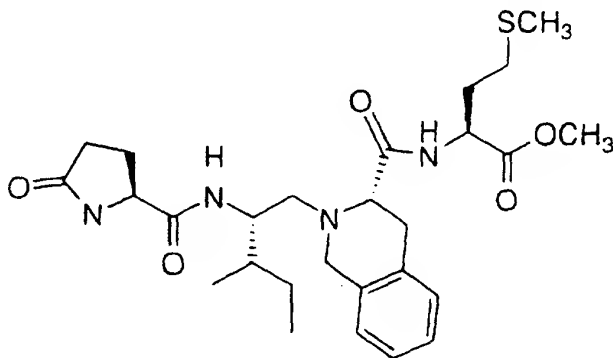
N-[1-Glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine
methyl ester;

10 N-[1-Glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine;

N-[L-Pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-
3(S)-isoquinolinecarbonyl-methionine

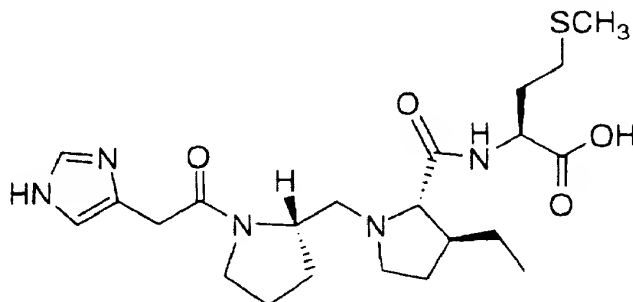


15 N-[L-Pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-
3(S)-isoquinolinecarbonyl-methionine methyl ester

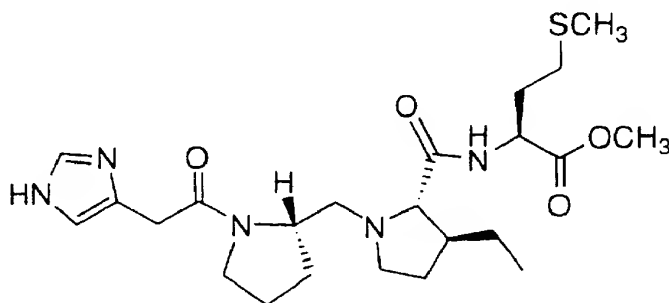


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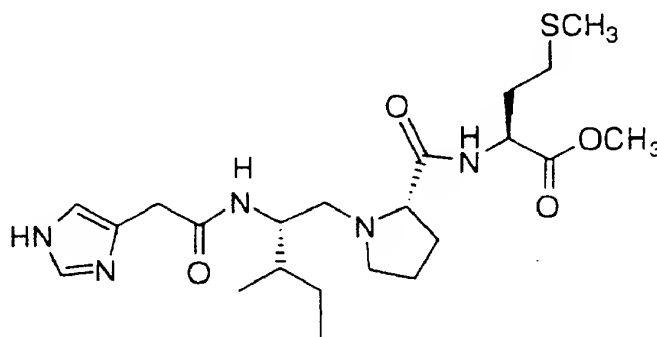
N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine



5 N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine methyl ester

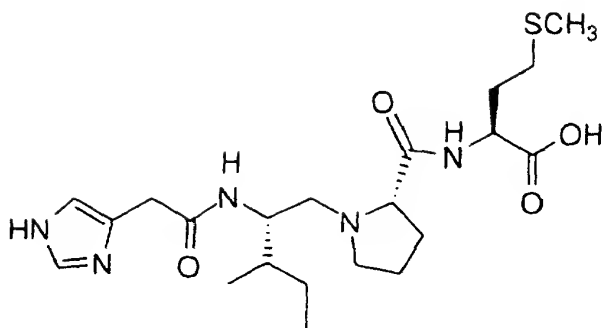


N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine methyl ester



10 N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine

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N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine methyl ester

- 5 N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine

N-[L-Pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine methyl ester

10

N-[L-Pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine

- 15 N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine methyl ester

N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine

- 20 N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-prolyl-methionine methyl ester

N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-prolyl-methionine

25

N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine methyl ester

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N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylpropyl-methionine

- 5 N-[1-Glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylpropyl-methionine methyl ester

N-[1-Glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylpropyl-methionine
2(S)-Butyl-1-(2,3-diaminoprop-1-yl)-1-(1-naphthoyl)piperazine

10

1-(3-Amino-2-(2-naphthylmethylamino)prop-1-yl)-2(S)-butyl-4-(1-naphthoyl)piperazine

- 15 2(S)-Butyl-1-{5-[1-(2-naphthylmethyl)]-4,5-dihydroimidazol}methyl-4-(1-naphthoyl)piperazine

1-[5-(1-Benzylimidazol)methyl]-2(S)-butyl-4-(1-naphthoyl)piperazine

- 20 1-[5-[1-(4-nitrobenzyl)]imidazolylmethyl]-2(S)-butyl-4-(1-naphthoyl)piperazine

1-(3-Acetamidomethylthio-2(R)-aminoprop-1-yl)-2(S)-butyl-4-(1-naphthoyl)piperazine

- 25 2(S)-Butyl-1-[2-(1-imidazolyl)ethyl]sulfonyl-4-(1-naphthoyl)piperazine

2(R)-Butyl-1-imidazolyl-4-methyl-4-(1-naphthoyl)piperazine

- 30 2(S)-Butyl-4-(1-naphthoyl)-1-(3-pyridylmethyl)piperazine

1-2(S)-butyl-(2(R)-(4-nitrobenzyl)amino-3-hydroxypropyl)-4-(1-naphthoyl)piperazine

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1-(2(R)-Amino-3-hydroxyheptadecyl)-2(S)-butyl-4-(1-naphthoyl)-
piperazine

5 2(S)-Benzyl-1-imidazolyl-4-methyl-4-(1-naphthoyl)piperazine

1-(2(R)-Amino-3-(3-benzylthio)propyl)-2(S)-butyl-4-(1-
naphthoyl)piperazine

10 1-(2(R)-Amino-3-[3-(4-nitrobenzylthio)propyl])-2(S)-butyl-4-(1-
naphthoyl)piperazine

2(S)-Butyl-1-[(4-imidazolyl)ethyl]-4-(1-naphthoyl)piperazine

15 2(S)-Butyl-1-[(4-imidazolyl)methyl]-4-(1-naphthoyl)piperazine

2(S)-Butyl-1-[(1-naphth-2-ylmethyl)-1H-imidazol-5-yl]acetyl]-4-(1-
naphthoyl)piperazine

20 2(S)-Butyl-1-[(1-naphth-2-ylmethyl)-1H-imidazol-5-yl]ethyl]-4-(1-
naphthoyl)piperazine

1-(2(R)-Amino-3-hydroxypropyl)-2(S)-butyl-4-(1-naphthoyl)piperazine

25 1-(2(R)-Amino-4-hydroxybutyl)-2(S)-butyl-4-(1-naphthoyl)piperazine

1-(2-Amino-3-(2-benzyloxyphenyl)propyl)-2(S)-butyl-4-(1-
naphthoyl)piperazine

30 1-(2-Amino-3-(2-hydroxyphenyl)propyl)-2(S)-butyl-4-(1-
naphthoyl)piperazine

1-[3-(4-imidazolyl)propyl]-2(S)-butyl-4-(1-naphthoyl)-piperazine

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2(S)-*n*-Butyl-4-(2,3-dimethylphenyl)-1-(4-imidazolylmethyl)-
piperazin-5-one

5 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-
dimethylphenyl)piperazin-5-one

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-
2(S)-(2-methoxyethyl)piperazin-5-one

10 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(1-naphthylmethyl)imidazol-5-
ylmethyl]-piperazine

2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(2-naphthylmethyl)imidazol-5-
ylmethyl]-piperazine

15 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine

20 2(S)-*n*-Butyl-1-[1-(4-methoxybenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine

2(S)-*n*-Butyl-1-[1-(3-methyl-2-butenyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine

25 2(S)-*n*-Butyl-1-[1-(4-fluorobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine

2(S)-*n*-Butyl-1-[1-(4-chlorobenzyl)imidazol-5-ylmethyl]-4-(1-
naphthoyl)piperazine

30 1-[1-(4-Bromobenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-
naphthoyl)piperazine

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2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethylbenzyl)imidazol-5-ylmethyl]-piperazine

5 2(S)-*n*-Butyl-1-[1-(4-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine

2(S)-*n*-Butyl-1-[1-(3-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine

10 1-[1-(4-Phenylbenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-naphthoyl)-piperazine

15 2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(2-phenylethyl)imidazol-5-ylmethyl]-piperazine

2(S)-*n*-Butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethoxy)imidazol-5-ylmethyl]piperazine

20 1-[[1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetyl]-2(S)-*n*-butyl-4-(1-naphthoyl)piperazine
(N-[1-Cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine

25 (N-[1-Cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine methyl ester

(N-[1-Cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester
N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester
30

Compounds which are useful in the present invention, and methods of synthesis thereof, can be found in the following patents, pending applications and publications:

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- USSN 60/005,059 filed on October 6, 1995;
USSN 60/005,063 filed on October 6, 1995
USSN 60/005,521 filed on October 13, 1995
WO 95/32987 published on 7 December 1995.
- 5 U. S. Pat. No. 5,420,245;
European Pat. Publ. 0 618 221 ;
WO 94/26723;
WO 95/08542 ;
WO 95/11917;
- 10 WO 95/12612.
- U. S. Pat. No. 5,238,922 granted on August 24, 1993; ;
- 15 U. S. Pat. No. 5,340,828 granted on August 23, 1994; ;
U. S. Pat. No. 5,352,705 granted on October 4, 1994;
U. S. Pat. No. 5,326,773 granted on July 5, 1994;
- 20 USSN 07/968,022 filed on October 29, 1992 ;
- USSN 08/968,025 filed on October 29, 1992 and USSN 08/143,943 filed
on October 27, 1993 ;
- 25 USSN 08/080,028 filed on June 18, 1993 and USSN 08/237,586 filed on
May 11, 1994 ;
- USSN 08/314,987 filed on September 29, 1994
- 30 USSN 08/315,171 filed on September 29, 1994
- USSN 08/315,046 filed on September 29, 1994 ;
- 35 USSN 08/315,161 filed on September 29, 1994; USSN 08/399,282 filed
on March 6, 1995; USSN 472,077 filed on June 6, 1995 and USSN
08/527,972 filed on September 14, 1995

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USSN 08/315,151 filed on September 29, 1994 ;

USSN 08/314,974 filed on September 29, 1994

5

USSN 08/412,621 filed on March 29, 1995 and USSN 08/448,865 filed
on May 24, 1995 ;

USSN 08/413,137 filed on March 29, 1995; ;

10

USSN 08/412,828 filed on March 29, 1995;

USSN 08/412,829 filed on March 29, 1995; and USSN 08/470,690 filed
on June 6, 1995;

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USSN 08/412,830 filed on March 29, 1995;

USSN 08/449,038 filed on May 24, 1995; ;

20 USSN 08/468,160 filed on June 6, 1995; ;

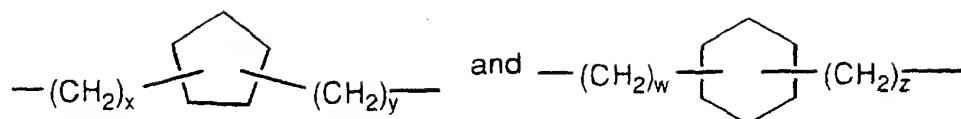
All patents, publications and pending patent applications identified are
hereby incorporated by reference.

25 The Raf antagonists are described herein using the terms
defined below unless otherwise specified.

The term "alkyl" refers to a monovalent alkane
(hydrocarbon) derived radical containing from 1 to 15 carbon atoms
unless otherwise defined. It may be straight, branched or cyclic.
Preferred straight or branched alkyl groups include methyl, ethyl,
30 propyl, isopropyl, butyl and t-butyl. Preferred cycloalkyl groups
include cyclopentyl and cyclohexyl.

Alkyl also includes a straight or branched alkyl group
which contains or is interrupted by a cycloalkylene portion.
Examples include the following:

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wherein: x and y = from 0-10; and w and z = from 0-9.

The alkylene and monovalent alkyl portion(s) of the alkyl group can be attached at any available point of attachment to the cycloalkylene portion.

When substituted alkyl is present, this refers to a straight, branched or cyclic alkyl group as defined above, substituted with 1-3 groups as defined with respect to each variable.

Heteroalkyl refers to an alkyl group having from 2-15 carbon atoms, and interrupted by from 1-4 heteroatoms selected from O, S and N.

The term "alkenyl" refers to a hydrocarbon radical straight, branched or cyclic containing from 2 to 15 carbon atoms and at least one carbon to carbon double bond. Preferably one carbon to carbon double bond is present, and up to four non-aromatic (non-resonating) carbon-carbon double bonds may be present. Examples of alkenyl groups include vinyl, allyl, isopropenyl, pentenyl, hexenyl, heptenyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, 1-propenyl, 2-butenyl, 2-methyl-2-butenyl, isoprenyl, farnesyl, geranyl, geranylgeranyl and the like. Preferred alkenyl groups include ethenyl, propenyl, butenyl and cyclohexenyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and may be substituted when a substituted alkenyl group is provided.

The term "alkynyl" refers to a hydrocarbon radical straight, branched or cyclic, containing from 2 to 15 carbon atoms and at least one carbon to carbon triple bond. Up to three carbon-carbon triple bonds may be present. Preferred alkynyl groups include ethynyl, propynyl and butynyl. As described above with respect to alkyl, the straight, branched or cyclic portion of the alkynyl group may contain triple bonds and may be substituted

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when a substituted alkynyl group is provided.

Aryl refers to aromatic rings e.g., phenyl, substituted phenyl and like groups as well as rings which are fused, e.g., naphthyl and the like. Aryl thus contains at least one ring having at least 6 atoms, with up to two such rings being present, containing up to 10 atoms therein, with alternating (resonating) double bonds between adjacent carbon atoms. The preferred aryl groups are phenyl and naphthyl. Aryl groups may likewise be substituted as defined below. Preferred substituted aryls include phenyl and naphthyl substituted with one or two groups. With regard to the farnesyl transferase inhibitors, "aryl" is intended to include any stable monocyclic, bicyclic or tricyclic carbon ring(s) of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of aryl groups include phenyl, naphthyl, anthracenyl, biphenyl, tetrahydronaphthyl, indanyl, phenanthrenyl and the like.

The term "heteroaryl" refers to a monocyclic aromatic hydrocarbon group having 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing at least one heteroatom, O, S or N, in which a carbon or nitrogen atom is the point of attachment, and in which one additional carbon atom is optionally replaced by a heteroatom selected from O or S, and in which from 1 to 3 additional carbon atoms are optionally replaced by nitrogen heteroatoms. The heteroaryl group is optionally substituted with up to three groups.

Heteroaryl thus includes aromatic and partially aromatic groups which contain one or more heteroatoms. Examples of this type are thiophene, purine, imidazopyridine, pyridine, oxazole, thiazole, oxazine, pyrazole, tetrazole, imidazole, pyridine, pyrimidine, pyrazine and triazine. Examples of partially aromatic groups are tetrahydroimidazo[4,5-c]pyridine, phthalidyl and saccharinyl, as defined below.

With regard to the farnesyl transferase inhibitors, the term heterocycle or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic or stable 11-15 membered tricyclic heterocycle ring which is either saturated or

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unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydro-benzothienyl, dihydrobenzothiopyranyl, dihydrobenzothio-pyranyl sulfone, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyridyl N-oxide, pyridonyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinolinyl N-oxide, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydro-quinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl. Preferably, heterocycle is selected from imidazolyl, 2-oxopyrrolidinyl, piperidyl, pyridyl and pyrrolidinyl.

Substituted alkyl, aryl and heteroaryl, and the substituted portions of aralkyl, aralkoxy, heteroaralkyl, heteroaralkoxy and like groups are substituted with from 1-3 groups selected from the group consisting of: halo, hydroxy, cyano, acyl, acylamino, aralkoxy, alkylsulfonyl, arylsulfonyl, alkylsulfonylamino, arylsulfonylamino, alkylaminocarbonyl, alkyl, alkoxy, aryl, aryloxy, aralkoxy, amino, alkylamino, dialkylamino, and sulfonylamino.

With regard to the farnesyl transferase inhibitors, the terms "substituted aryl", "substituted heterocycle" and "substituted cycloalkyl" are intended to include the cyclic group which is substituted with 1 or

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2 substituents selected from the group which includes but is not limited to F, Cl, Br, CF₃, NH₂, N(C₁-C₆ alkyl)₂, NO₂, CN, (C₁-C₆ alkyl)O-, -OH, (C₁-C₆ alkyl)S(O)_m-, (C₁-C₆ alkyl)C(O)NH-, H₂N-C(NH)-, (C₁-C₆ alkyl)C(O)-, (C₁-C₆ alkyl)OC(O)-, N₃, (C₁-C₆ alkyl)OC(O)NH- and C₁-C₂₀ alkyl.

The terms "heterocycloalkyl" and "heterocyclyl" refer to a cycloalkyl group (nonaromatic) in which one of the carbon atoms in the ring is replaced by a heteroatom selected from O, S(O)_y or N, and in which up to three additional carbon atoms may be replaced by said heteroatoms. When three heteroatoms are present in the heterocycle, they are not all linked together.

Examples of heterocyclyls are piperidinyl, morpholinyl, pyrrolidinyl, tetrahydrofuranyl, imidazolynyl, piperazinyl, pyrrolidine-2-one, piperidine-2-one and the like.

Acyl as used herein refers to -C(O)C₁₋₆ alkyl and -C(O)-aryl.

Acylamino refers to the group -NHC(O)C₁₋₆ alkyl and -NHC(O)aryl.

Aralkoxy refers to the group -OC₁₋₆ alkylaryl.

Alkylsulfonyl refers to the group -SO₂C₁₋₆ alkyl.

Alkylsulfonylamino refers to the group -NHSO₂C₁₋₆alkyl.

Arylsulfonylamino refers to the group -NHSO₂aryl.

Alkylaminocarbonyl refers to the group -C(O)NHC₁₋₆ alkyl.

Aryloxy refers to the group -O-aryl.

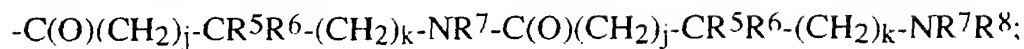
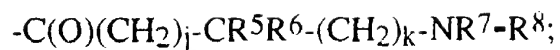
Aralkoxy refers to the group -O-C₁₋₆ alkylaryl.

Sulfonylamino refers to the group -NHSO₃H.

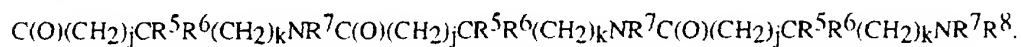
Halo means Cl, F, Br and I selected on an independent basis.

Within -[C(O)(CH₂)_j-CR⁵R⁶-(CH₂)_k-NR⁷]_p-R⁸, there may be from 1 to 3 groups -[C(O)(CH₂)_j-CR⁵R⁶-(CH₂)_k-NR⁷]-. Thus, -[C(O)(CH₂)_j-CR⁵R⁶-(CH₂)_k-NR⁷]_p-R⁸ with p equal to 1, 2 or 3 means the following:

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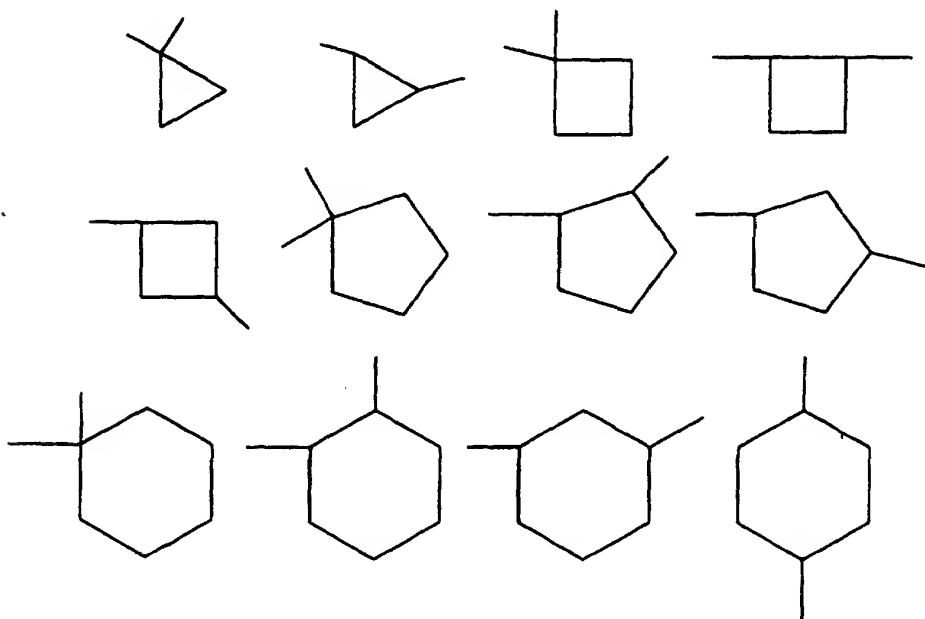


5 and



Within these groups, the variables are determined independently. For
 10 example, when more than one j is present, they may be the same or
 different. When CR^5R^6 is taken in combination, it represents a 3, 4,
 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or
 a heteroaryl group. Examples of suitable cycloalkylene attachment
 are as follows:

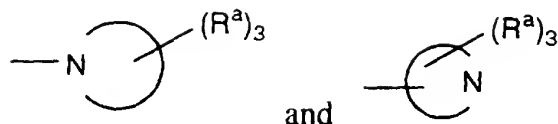
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In each of the patterns of attachment noted above, the ring may also be
 heterocyclic as defined above.

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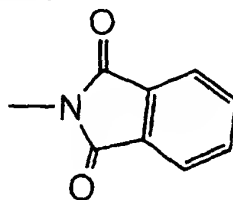
are optional substituents linked to the HETCy group.

5 and independently represent mono or bicyclic ring systems, non-aromatic or partially aromatic, containing from 5-10 ring atoms, 1-4 of which are N and 0-1 of which are O or S(O)_y, with y equal to 0, 1 or 2, and when partially aromatic, the non-aromatic portion thereof optionally containing 1-2 carbonyl groups. Hence, these
10 ring systems can be heteroaryl or heterocyclic as defined above.

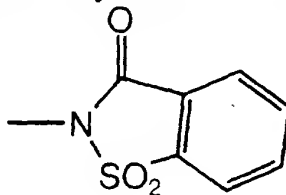
is linked to HETCy through a nitrogen atom contained in the ring system, either directly or through a linking group which is part of R'. Examples include phthalidyl and saccharinyl, as further defined below.

15 is likewise linked to HETCy, but through a carbon atom contained in the ring system.

The term phthalidyl refers to the heteroaryl group:



The term saccharinyl refers to the heteroaryl group:



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In the present method, amino acids which are disclosed are identified both by conventional 3 letter and single letter abbreviations as indicated below:

| | | | |
|----|---------------|-----|---|
| 5 | Alanine | Ala | A |
| | Arginine | Arg | R |
| | Asparagine | Asn | N |
| | Aspartic acid | Asp | D |
| | Asparagine or | | |
| 10 | Aspartic acid | Asx | B |
| | Cysteine | Cys | C |
| | Glutamine | Gln | Q |
| | Glutamic acid | Glu | E |
| | Glutamine or | | |
| 15 | Glutamic acid | Glx | Z |
| | Glycine | Gly | G |
| | Histidine | His | H |
| | Isoleucine | Ile | I |
| | Leucine | Leu | L |
| 20 | Lysine | Lys | K |
| | Methionine | Met | M |
| | Phenylalanine | Phe | F |
| | Proline | Pro | P |
| | Serine | Ser | S |
| 25 | Threonine | Thr | T |
| | Tryptophan | Trp | W |
| | Tyrosine | Tyr | Y |
| | Valine | Val | V |

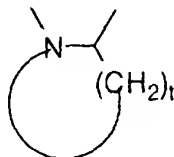
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The compounds used in the present method may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. Unless otherwise

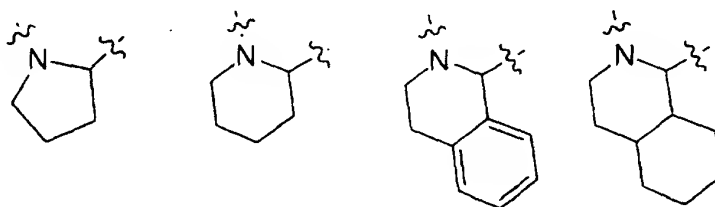
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specified, named amino acids are understood to have the natural "L" stereoconfiguration

The following structure:



- 5 represents a cyclic amine moiety having 5 or 6 members in the ring, such a cyclic amine which may be optionally fused to a phenyl or cyclohexyl ring. Examples of such a cyclic amine moiety include, but are not limited to, the following specific structures:



- 10 It is also understood that substitution on the cyclic amine moiety by R^{2a} and R^{2b} may be on different carbon atoms or on the same carbon atom. When R^3 and R^4 are combined to form $-(CH_2)_s-$, cyclic moieties are formed. Examples of such cyclic moieties include, but are not limited to:

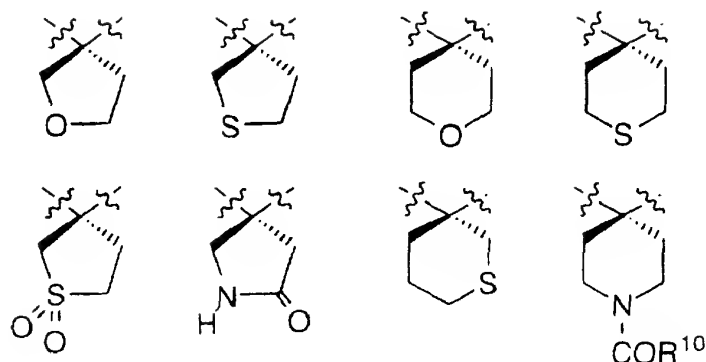


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When R^{5a} and R^{5b} are combined to form $-(CH_2)_s-$, cyclic moieties as described hereinabove for R^3 and R^4 are formed. In addition, such cyclic moieties may optionally include a heteroatom(s). Examples of such heteroatom-containing cyclic moieties include, but are not limited to:

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The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenyl-acetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

It is intended that the definition of any substituent or variable (e.g., R¹⁰, Z, n, etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus, -N(R¹⁰)₂ represents -NHH, -NHCH₃, -NHC₂H₅, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth below.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared by reacting the free base with stoichiometric amounts or with an excess of the desired salt-

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forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

The compounds of formulas (II-a) through (II-k) can be synthesized from their constituent amino acids by conventional peptide synthesis techniques, and the additional methods described below. Standard methods of peptide synthesis are disclosed, for example, in the following works: Schroeder *et al.*, "The Peptides", Vol. I, Academic Press 1965, or Bodanszky *et al.*, "Peptide Synthesis", Interscience Publishers, 1966, or McOmie (ed.) "Protective Groups in Organic Chemistry", Plenum Press, 1973, or Barany *et al.*, "The Peptides: Analysis, Synthesis, Biology" 2, Chapter 1, Academic Press, 1980, or Stewart *et al.*, "Solid Phase Peptide Synthesis", Second Edition, Pierce Chemical Company, 1984. Also useful in exemplifying syntheses of specific unnatural amino acid residues are European Pat. Appl. No. 0 350 163 A2 (particularly page 51-52) and J. E. Baldwin *et al.* *Tetrahedron*, 50:5049-5066 (1994). With regards to the synthesis of instant compounds containing a (β -acetylamino)alanine residue at the C-terminus, use of the commercially available N α -Z-L-2,3-diaminopropionic acid (Fluka) as a starting material is preferred.

Abbreviations used in the description of the chemistry and in the Examples that follow are:

| | |
|-------------------|---|
| Ac ₂ O | Acetic anhydride; |
| Boc | t-Butoxycarbonyl; |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene; |
| DMAP | 4-Dimethylaminopyridine; |
| DME | 1,2-Dimethoxyethane; |
| DMF | Dimethylformamide; |
| EDC | 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide- |
| | hydrochloride; |
| HOBT | 1-Hydroxybenzotriazole hydrate; |
| Et ₃ N | Triethylamine; |
| EtOAc | Ethyl acetate; |
| FAB | Fast atom bombardment; |

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| | |
|----------|---|
| HOObt | 3-Hydroxy-1,2,2-benzotriazin-4(3H)-one; |
| HPLC | High-performance liquid chromatography; |
| MCPBA | m-Chloroperoxybenzoic acid; |
| MsCl | Methanesulfonyl chloride; |
| 5 NaHMDS | Sodium bis(trimethylsilyl)amide; |
| Py | Pyridine; |
| TFA | Trifluoroacetic acid; |
| THF | Tetrahydrofuran. |

10 The compounds of formula (I-a) and (I-b) are prepared in accordance with U. S. Application No. 60/005,059 filed on October 6, 1995 and 60/005,063 filed on October 6, 1995. Two general methods for preparation of the imidazole nucleus are outlined. In the first, a suitably protected picolyl alcohol is deprotonated with
15 a strong base such as *n*-butyl lithium or lithium diisopropyl amide and the resulting anion is reacted with an appropriate *N,O*-dimethylhydrox- amide to give a protected alpha hydroxy ketone. The protected alpha hydroxy ketone is then condensed with a suitably functionalized and protected aminoaldehyde in the presence of ammonium acetate, acetic
20 acid and copper acetate.

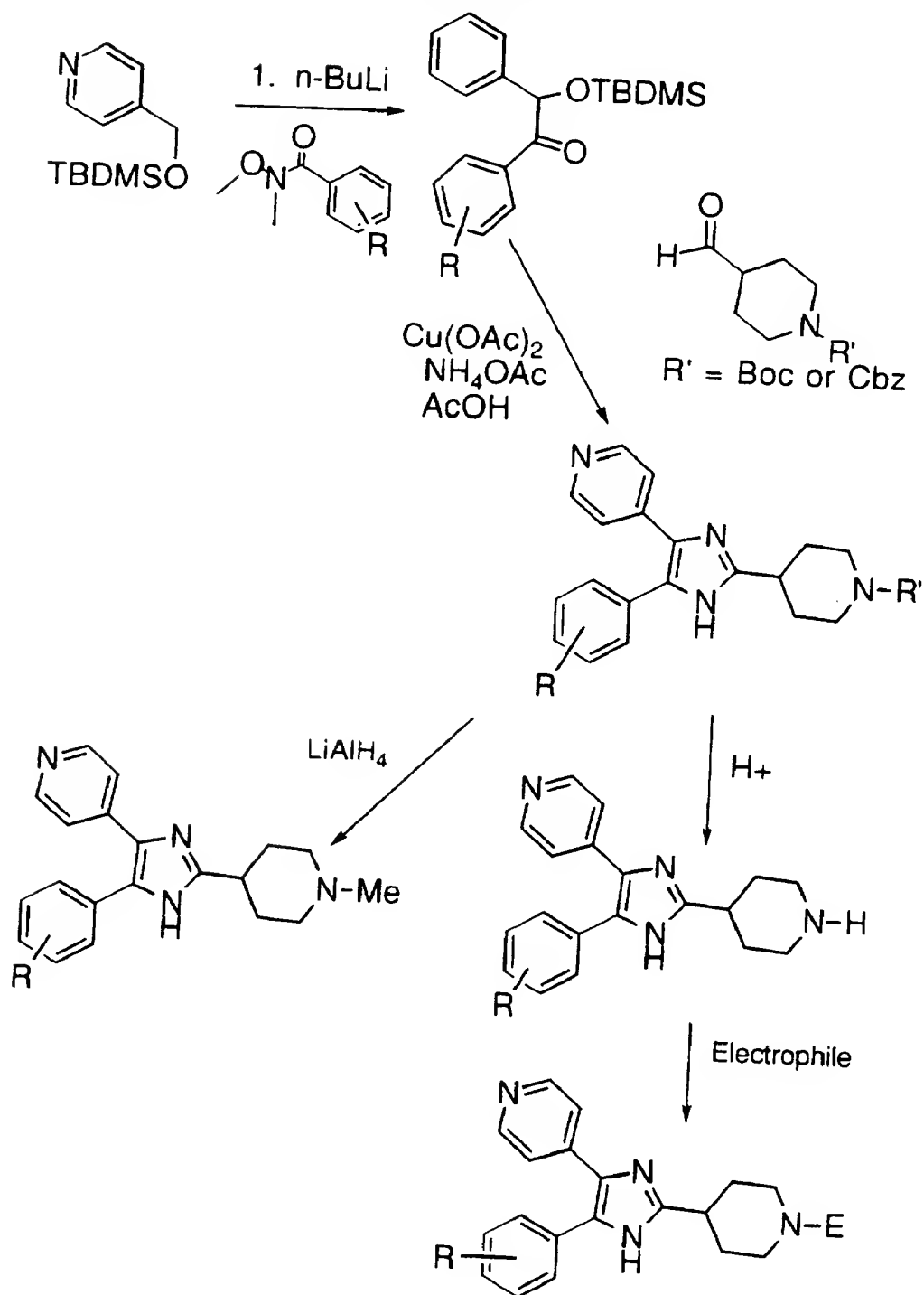
 The aldehydes typically used contain a suitably protected nitrogen atom. After the imidazole nucleus has been formed, the nitrogen is deprotected and then reacted with an appropriate electrophilic reagent to provide the final compounds.

25 In the second method, a suitably protected picolyl alcohol is deprotonated with a strong base such as *n*-butyl lithium or lithium diisopropyl amide and the resulting anion is reacted with an appropriate aryl or alkyl aldehyde to give a mono-protected diol. The protecting group is removed and the resulting diol is oxidized (by the method
30 of Swern or Moffat) to a dione. The dione is then condensed with a suitably functionalized and protected aminoaldehyde in the presence of ammonium acetate in acetic acid to give the imidazole.

 In this same manner, the nitrogen is deprotected and then
35 reacted with an appropriate electrophilic reagent to provide the compounds of formula I.

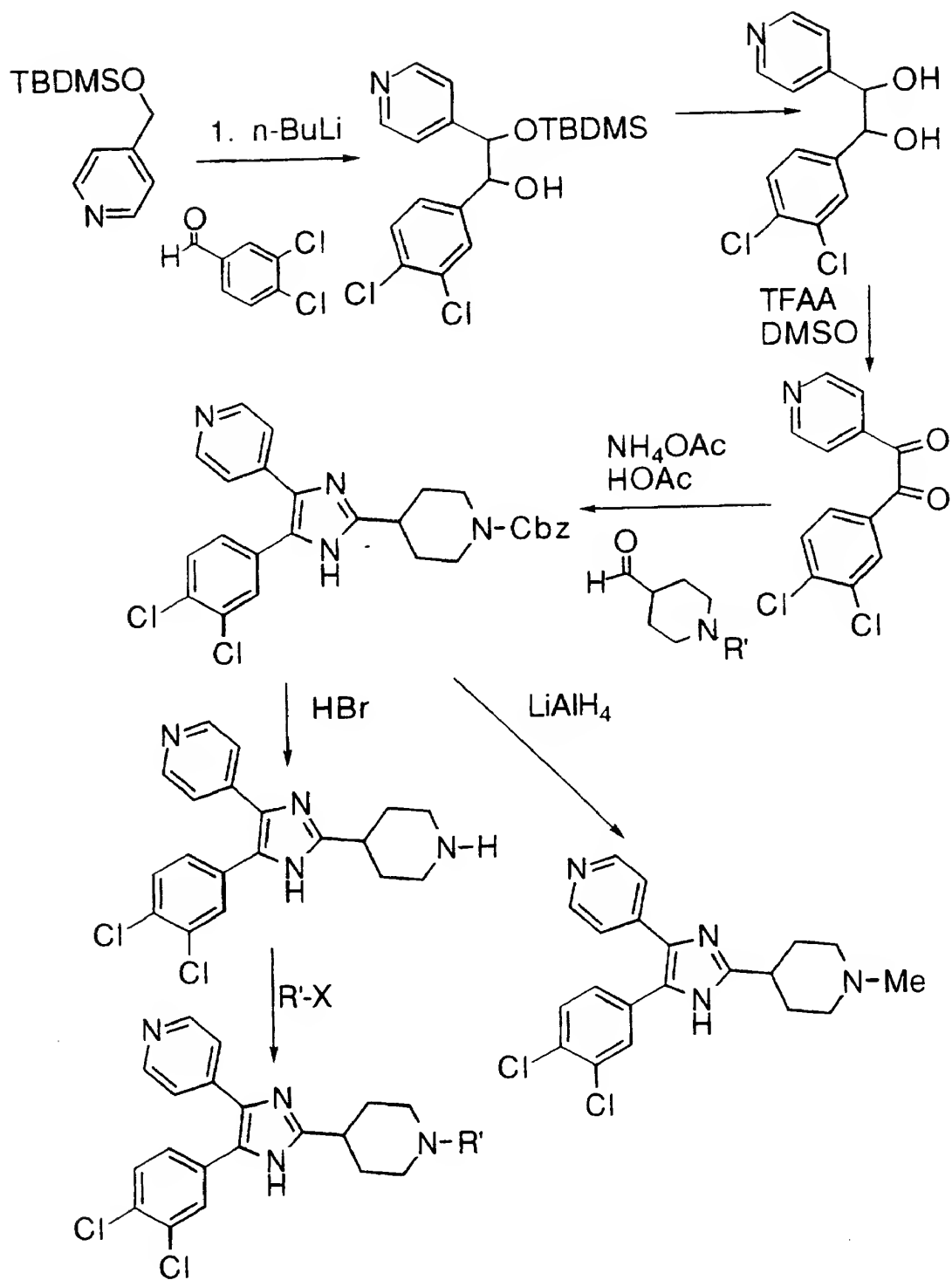
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Scheme 1



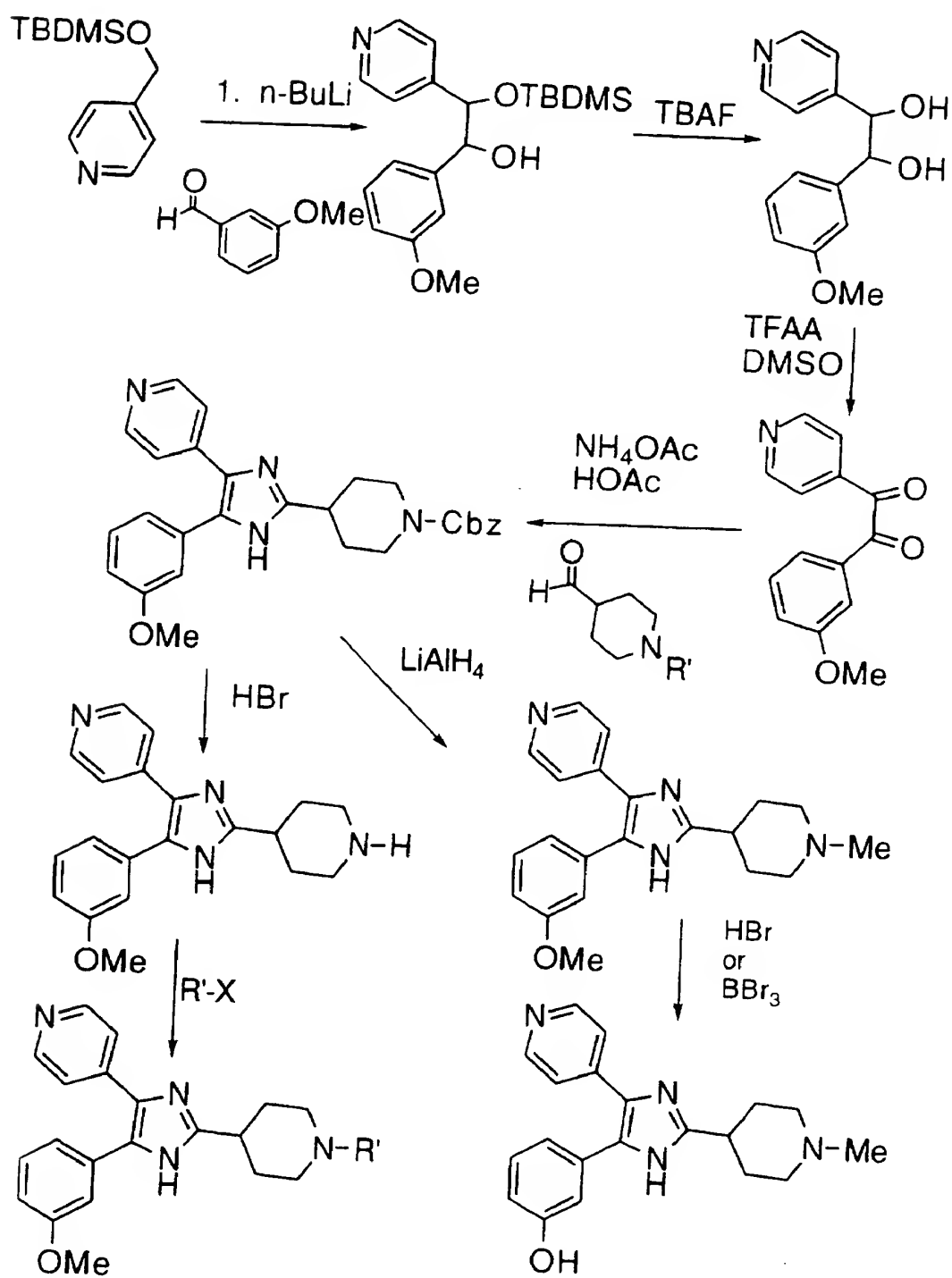
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Scheme 2



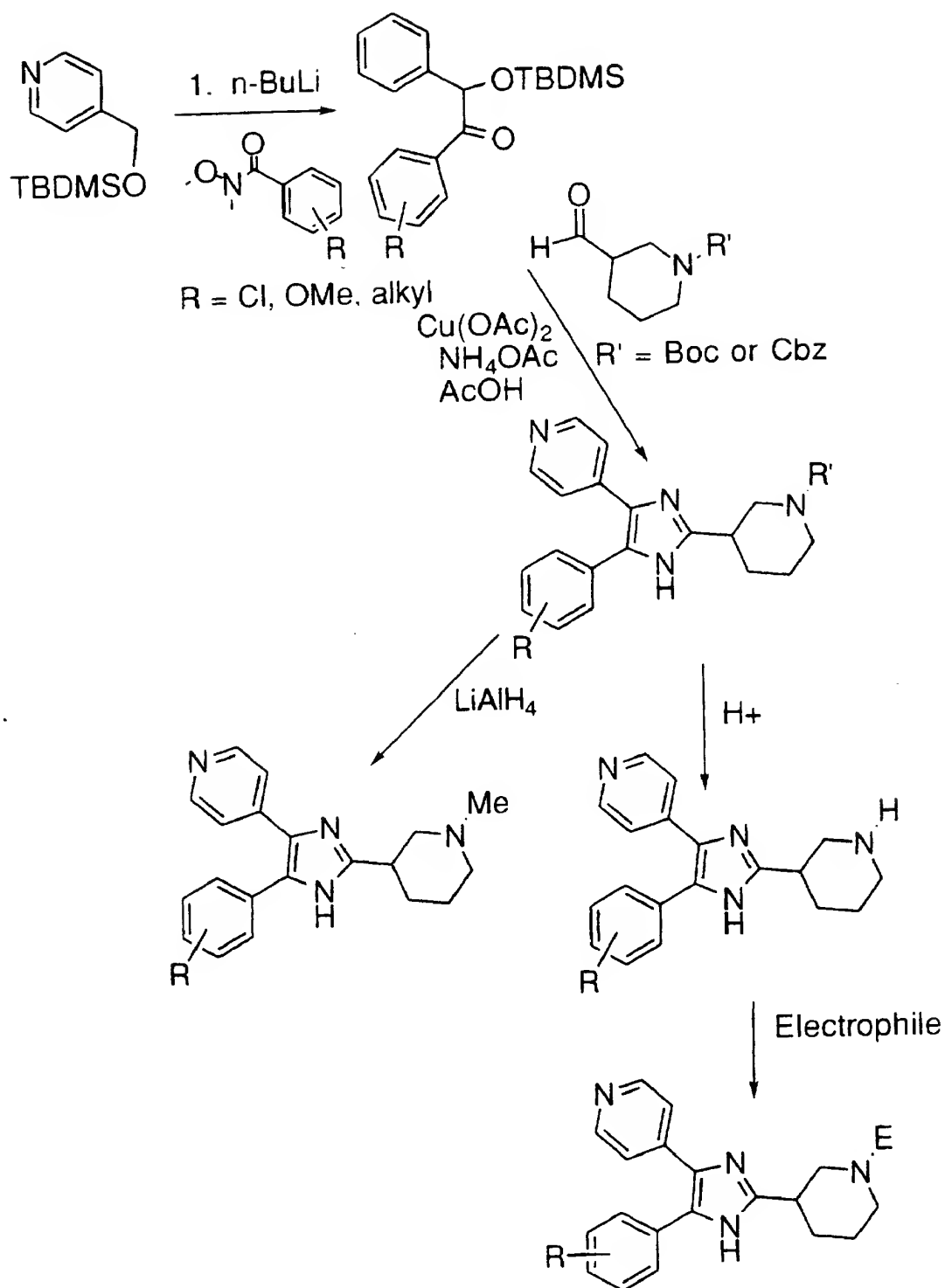
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Scheme 3



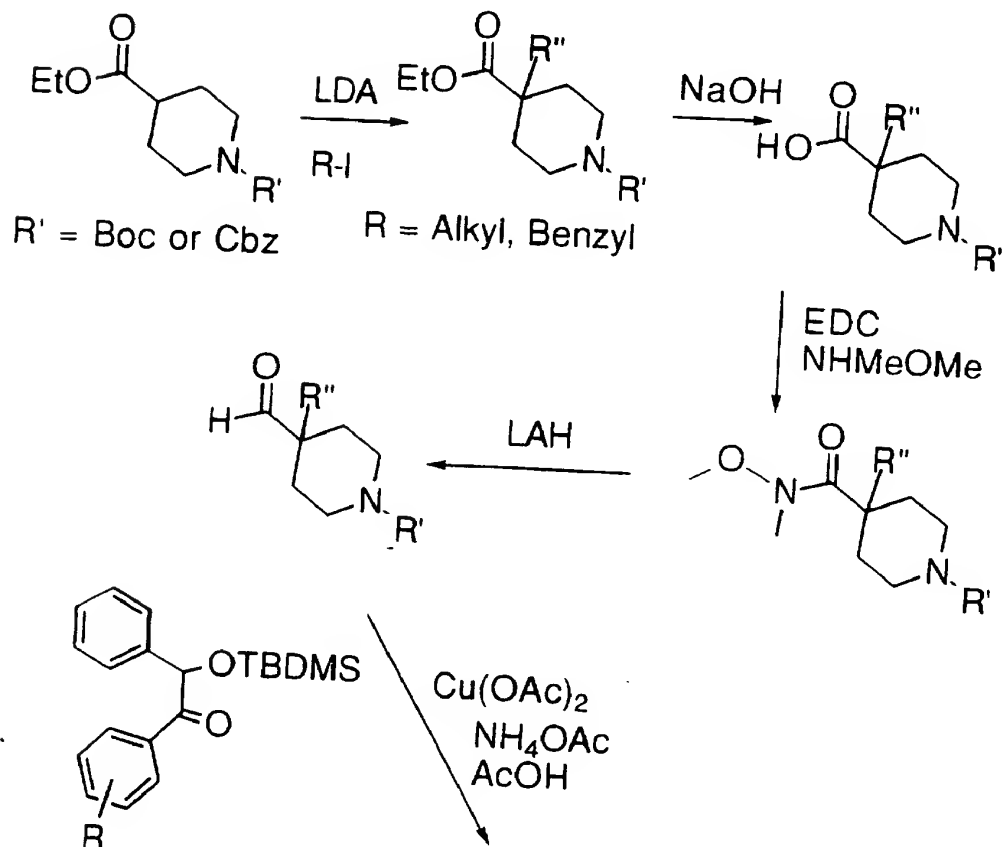
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Scheme 4

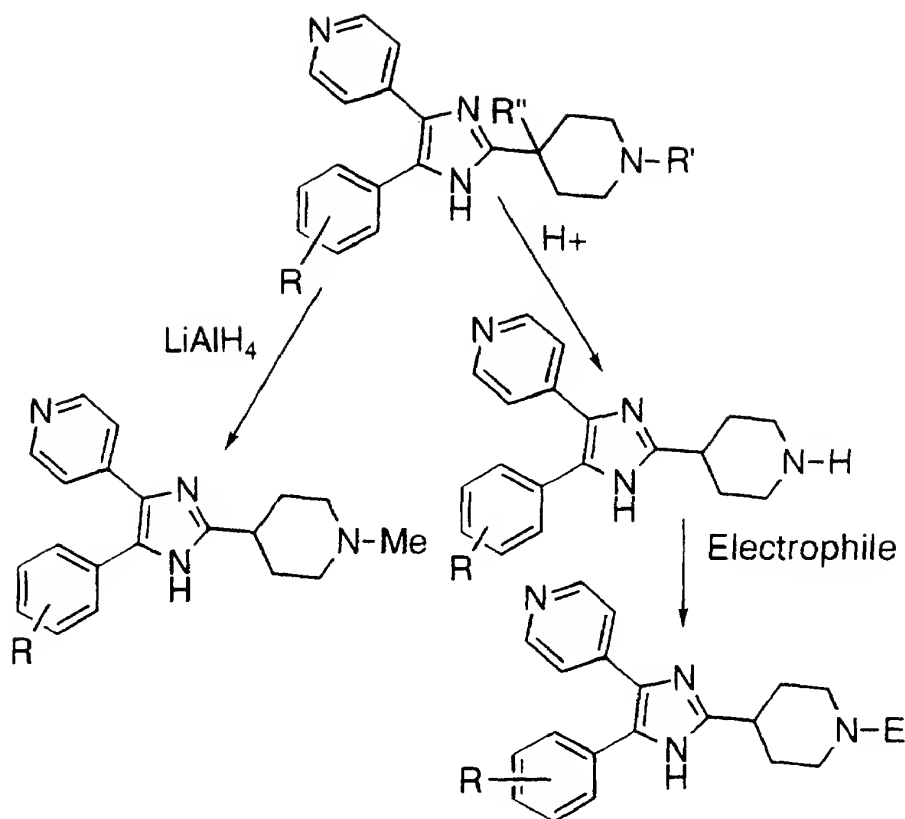


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Scheme 5



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TBDMSO refers to t-butyldimethylsilyloxy, TFAA refers to trifluoroacetic anhydride, TBDMS refers to t-butyldimethylsilyl,
 5 TBAF refers to tetrabutyl ammonium fluoride, Cbz refers to carboxyl-benzyl, Ac refers to acetyl, and LDA refers to lithium diisopropyl amide.

E represents an electrophile attached to the heterocyclic ring nitrogen atom. Examples of suitable electrophiles include alkyl
 10 halides, alkyl triflates, alkyl mesylates, benzyl halides, vinyl pyridine and the like. Hence, E represents alkyl, benzyl, vinyl and the like.

The compounds are useful in various pharmaceutically acceptable salt forms. The term "pharmaceutically acceptable salt" refers to those salt forms which would be apparent to the pharmaceutical chemist. i.e., those which are substantially non-toxic and which
 15 provide the desired pharmacokinetic properties, palatability, absorption,

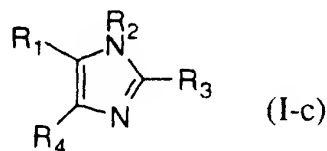
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distribution, metabolism or excretion. Other factors, more practical in nature, which are also important in the selection, are cost of the raw materials, ease of crystallization, yield, stability, hygroscopicity and flowability of the resulting bulk drug. Conveniently, pharmaceutical compositions may be prepared from the active ingredients in combination with pharmaceutically acceptable carriers.

Pharmaceutically acceptable salts include conventional non-toxic salts or quarternary ammonium salts formed, e.g., from non-toxic inorganic or organic acids. Non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methane-sulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the present invention can be synthesized by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid or base, in a suitable solvent or solvent combination.

Compounds of formula (I-c)



may be prepared using procedures described in PCT/US94/08297 published on 2 February 1995 and in U.S. Application No. 60/005,521 filed on October 13, 1995. Suitable procedures are also described in U.S. Patent Nos. 3,707,475 and 3,940,486.

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The Raf antagonists described herein are useful in various pharmaceutically acceptable salt forms. The term "pharmaceutically acceptable salt" refers to those salt forms which would be apparent to the pharmaceutical chemist. i.e., those which are substantially non-toxic and which provide the desired pharmacokinetic properties, palatability, absorption, distribution, metabolism or excretion. Other factors, more practical in nature, which are also important in the selection, are cost of the raw materials, ease of crystallization, yield, stability, hygroscopicity and flowability of the resulting bulk drug. Conveniently, pharmaceutical compositions may be prepared from the active ingredients in combination with pharmaceutically acceptable carriers.

Pharmaceutically acceptable salts include conventional non-toxic salts or quarternary ammonium salts formed, e.g., from non-toxic inorganic or organic acids. Non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methane-sulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts can be synthesized by conventional chemical methods. Generally, the salts are prepared by reacting the free base or acid with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid or base, in a suitable solvent or solvent combination.

The farnesyl transferase inhibitors of formula (II-a) through (II-c) can be synthesized in accordance with reaction schemes 1-16, in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures. Substituents R^a and R^b , as shown in the Schemes, represent the substituents R^2 , R^3 , R^4 , and R^5 ; however their point of attachment to the ring is illustrative only and is not meant to be limiting.

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These reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Reaction Schemes.

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Synopsis of reaction Schemes 1-16:

The requisite intermediates are in some cases commercially available, or can be prepared according to literature procedures, for the most part. In Scheme 1, for example, the synthesis of 2-alkyl substituted piperazines is outlined, and is essentially that described by J. S. Kiely and S. R. Priebe in Organic Preparations and Proceedings Int., **1990**, 22, 761-768. Boc-protected amino acids I, available commercially or by procedures known to those skilled in the art, can be coupled to N-benzyl amino acid esters using a variety of dehydrating agents such as DCC (dicyclohexycarbodiimide) or EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) in a solvent such as methylene chloride, chloroform, dichloroethane, or in dimethylformamide. The product II is then deprotected with acid, for example hydrogen chloride in chloroform or ethyl acetate, or trifluoroacetic acid in methylene chloride, and cyclized under weakly basic conditions to give the diketopiperazine III. Reduction of III with lithium aluminum hydride in refluxing ether gives the piperazine IV, which is protected as the Boc derivative V. The N-benzyl group can be cleaved under standard conditions of hydrogenation, e.g., 10% palladium on carbon at 60 psi hydrogen on a Parr apparatus for 24-48 h. The product VI can be treated with an acid chloride, or a carboxylic acid under standard dehydrating conditions to furnish the carboxamides VII. A final acid deprotection step gives the intermediate VIII (Scheme 2). Intermediate VIII can be reductively alkylated with a variety of aldehydes, such as IX, prepared by standard procedures, such as that described by O. P. Goel, U. Krolls, M. Stier and S. Kesten in Organic Syntheses, **1988**, 67, 69-75, from the appropriate amino acid (Scheme 3). The reductive alkylation can be accomplished at pH 5-7 with a variety of reducing agents, such as sodium triacetoxyborohydride

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or sodium cyanoborohydride, in a solvent such as dichloroethane, methanol or dimethylformamide. The product X can be deprotected to give the final compounds XI with trifluoroacetic acid in methylene chloride. The final product XI is isolated in the salt form, for example, as a trifluoroacetate, hydrochloride or acetate salt, among others. The product diamine XI can further be selectively protected to obtain XII, which can subsequently be reductively alkylated with a second aldehyde to obtain XIII. Removal of the protecting group, and conversion to the cyclized product such as the dihydroimidazole XV, can be accomplished by literature procedures.

Alternatively, the protected piperazine intermediate VII can be reductively alkylated with other aldehydes such as 1-trityl-4-carboxaldehyde or 1-trityl-4-imidazolylacetaldehyde, to give products such as XVI (Scheme IV) (Tr = trityl). The trityl protecting group can be removed from XVI to give XVII, or alternatively, XVI can first be treated with an alkyl halide then subsequently deprotected to give the alkylated imidazole XVIII. Alternatively, the intermediate VIII can be acylated or sulfonylated by standard techniques. The imidazole acetic acid XIX can be converted to the acetate XXI by standard procedures, and XXI can be first reacted with an alkyl halide, then treated with refluxing methanol to provide the regiospecifically alkylated imidazole acetic acid ester XXII. Hydrolysis and reaction with piperazine VIII in the presence of condensing reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) leads to acylated products such as XXIV.

If the piperazine VIII is reductively alkylated with an aldehyde which also has a protected hydroxyl group, such as XXV in Scheme 6, the protecting groups can be subsequently removed to unmask the hydroxyl group (Schemes 6, 7). The alcohol can be oxidized under standard conditions to *e.g.* an aldehyde, which can then be reacted with a variety of organometallic reagents such as Grignard reagents, to obtain secondary alcohols such as XXIX. In addition, the fully deprotected amino alcohol XXX can be reductively alkylated (under conditions described previously) with a variety of aldehydes to obtain secondary amines, such as XXXI (Scheme 7), or tertiary amines.

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The protected amino alcohol XXVII can also be utilized to synthesize 2-aziridinylmethylpiperazines such as XXXII (Scheme 8).

Treating XXVII with 1,1'-sulfonyldiimidazole and sodium hydride in a solvent such as dimethylformamide leads to the formation of aziridine
5 XXXII. The aziridine reacts in the presence of a nucleophile, such as a thiol, in the presence of base to yield the ring-opened product XXXIII.

Piperazine VIII can be reacted with an aldehyde derived from an amino acid, such as an O-alkylated tyrosine, to obtain compounds such as XXXIX. When R' is an aryl group, XXXIX can
10 first be hydrogenated to unmask the phenol, and the amine group deprotected with acid to produce XL. Alternatively, the amine protecting group in XXXIX can be removed, and O-alkylated phenolic amines such as XLI produced.

Depending on the identity of the amino acid I, various side
15 chains can be incorporated onto the piperazine. For example, when I is a protected β -benzyl ester of aspartic acid, the intermediate diketopiperazine XLII (where $n=1$ and $R=\text{benzyl}$) is obtained, as shown in Scheme 10. Subsequent reduction reduces the ester to the alcohol
XLIII, which can then be reacted with a variety of alkylating agents
20 such as an alkyl iodide, under basic conditions, for example, sodium hydride in dimethylformamide or tetrahydrofuran. The resulting ether XLIV can then be carried on to final products as described in Schemes 3-9.

N-Aryl piperazines can be prepared as described in Scheme
25 11. An aryl amine XLV is reacted with *bis*-chloroethyl amine hydrochloride (XLVI) in refluxing *n*-butanol to furnish compounds XLVII. The resulting piperazines XLVII can then be carried on to final products as described in Schemes 3-9.

Piperazin-5-ones can be prepared as shown in Scheme 12.
30 Reductive amination of protected amino aldehydes XLIX (prepared from I as described previously) gives rise to compound L. This is then reacted with bromoacetyl bromide under Schotten-Baumann conditions. Ring closure is effected with a base, such as sodium hydride, in a polar

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aprotic solvent, such as dimethylformamide, to give LI. The carbamate protecting group is removed under acidic conditions, such as trifluoroacetic acid in methylene chloride or hydrogen chloride gas in methanol or ethyl acetate, and the resulting piperazine can then be carried on to final products as described in Schemes 3-9.

The isomeric piperazin-3-ones can be prepared as described in Scheme 13. The imine formed from arylcarboxamides LII and 2-aminoglycinal diethyl acetal (LIII) can be reduced under a variety of conditions, including sodium triacetoxymethylborohydride in dichloroethane, to give the amine LIV. Amino acids I can be coupled to amines LIV under standard conditions, and the resulting amide LV when treated with aqueous acid in tetrahydrofuran can cyclize to the unsaturated LVI. Catalytic hydrogenation under standard conditions gives the requisite intermediate LVII, which is elaborated to final products as described in Schemes 3-9.

Access to alternatively substituted piperazines is described in Scheme 14. Following deprotection, e.g., with trifluoroacetic acid, the N-benzyl piperazine V can be acylated with an aryl carboxylic acid. The resulting N-benzyl aryl carboxamide LIX can be hydrogenated in the presence of a catalyst to give the piperazine carboxamide LX which can then be carried on to final products as described in Schemes 3-9.

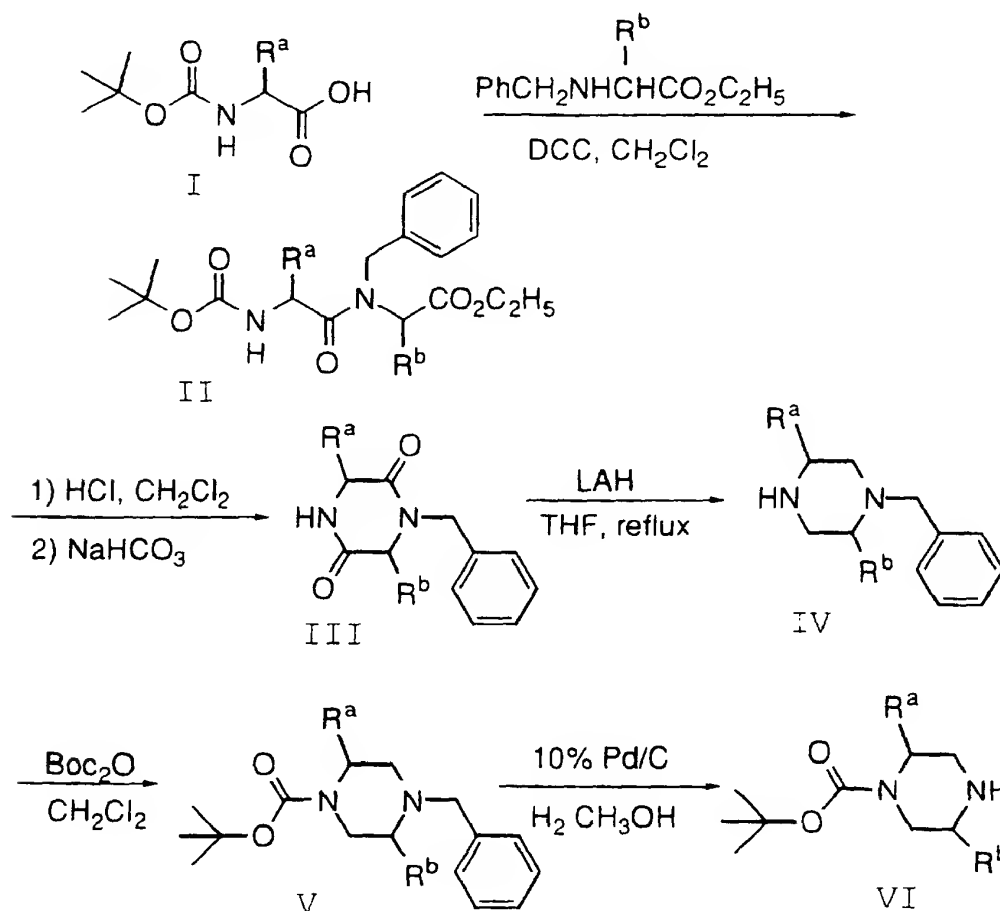
Reaction Scheme 15 provides an example of the synthesis of compounds wherein the substituents R^2 and R^3 are combined to form $-(CH_2)_u-$. For example, 1-aminocyclohexane-1-carboxylic acid LXI can be converted to the spiropiperazine LXVI essentially according to the procedures outlined in Schemes 1 and 2. The piperazine intermediate LXIX can be deprotected as before, and carried on to final products as described in Schemes 3-9. It is understood that reagents utilized to provide the substituent Y which is 2-(naphthyl) and the imidazolylalkyl substituent may be readily replaced by other reagents well known in the art and readily available to provide other N-substituents on the piperazine.

The aldehyde XLIX from Scheme 12 can also be reductively alkylated with an aniline as shown in Scheme 16. The

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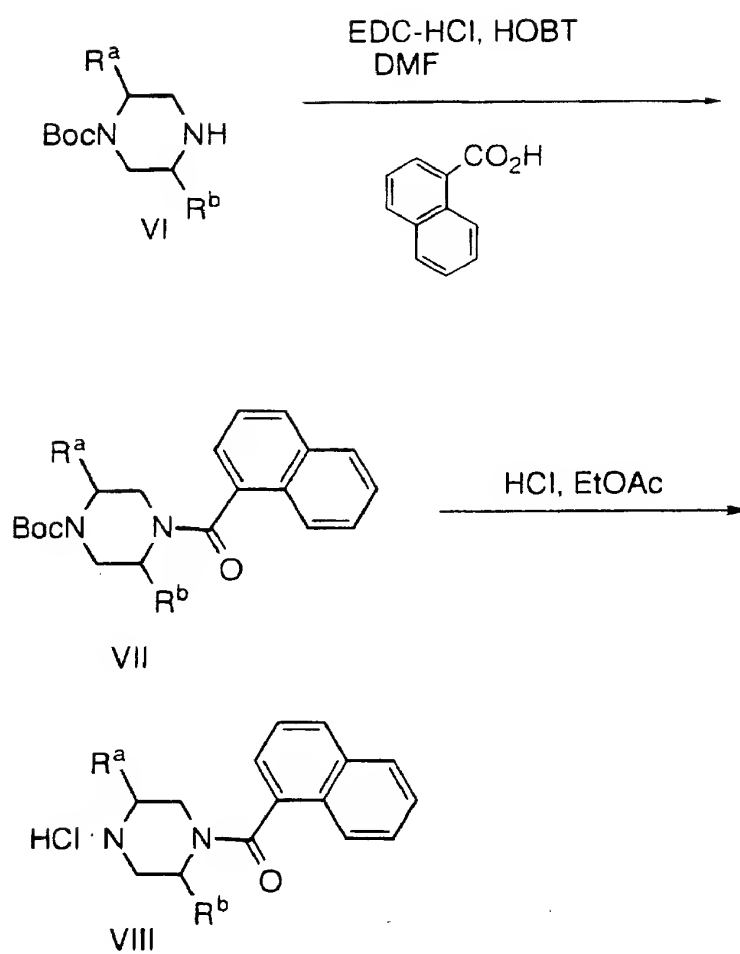
- product LXXI can be converted to a piperazinone by acylation with chloroacetyl chloride to give LXXII, followed by base-induced cyclization to LXXIII. Deprotection, followed by reductive alkylation with a protected imidazole carboxaldehyde leads to LXXV, which can be alkylated with an arylmethylhalide to give the imidazolium salt LXXVI. Final removal of protecting groups by either solvolysis with a lower alkyl alcohol, such as methanol, or treatment with triethylsilane in methylene chloride in the presence of trifluoroacetic acid gives the final product LXXVII.

SCHEME 1

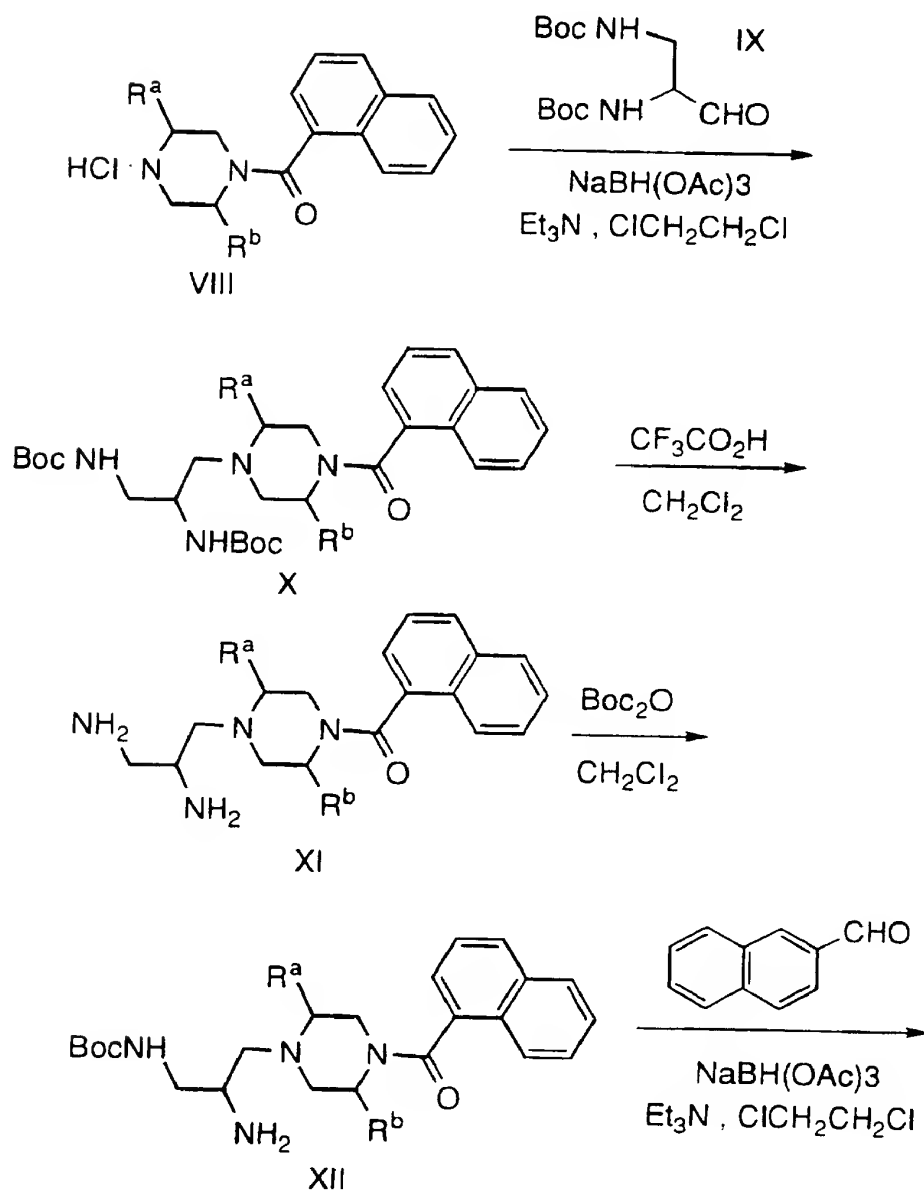


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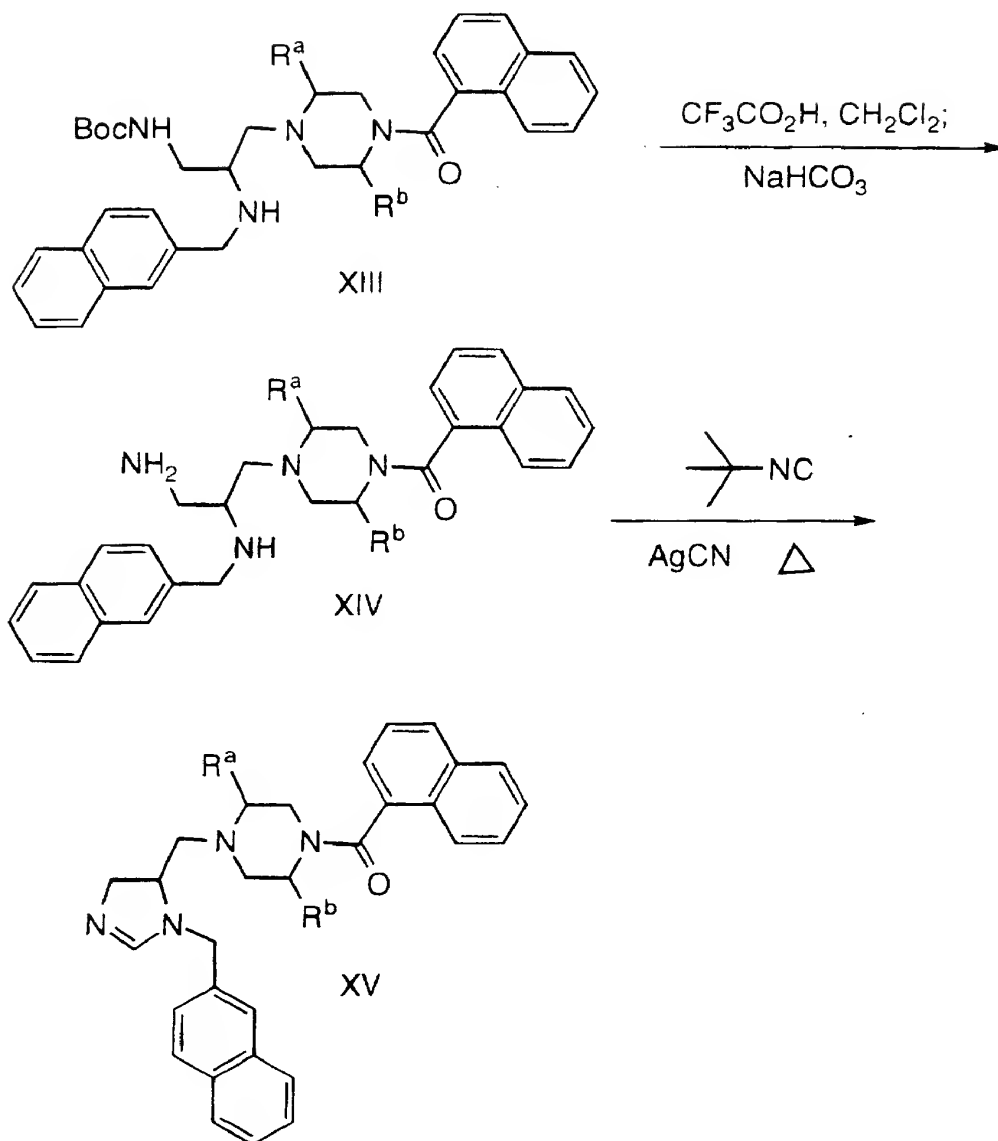
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SCHEME 2

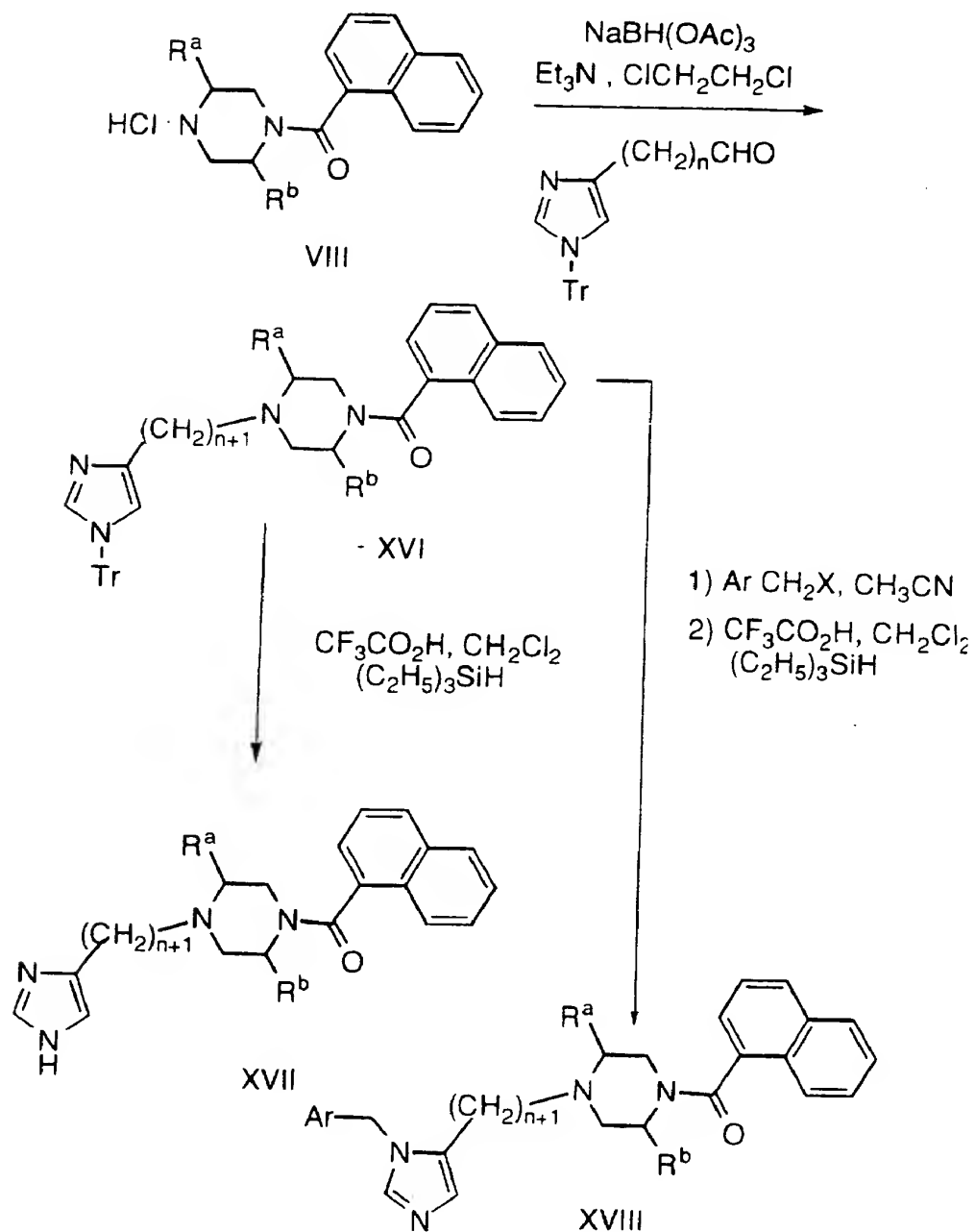
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SCHEME 3

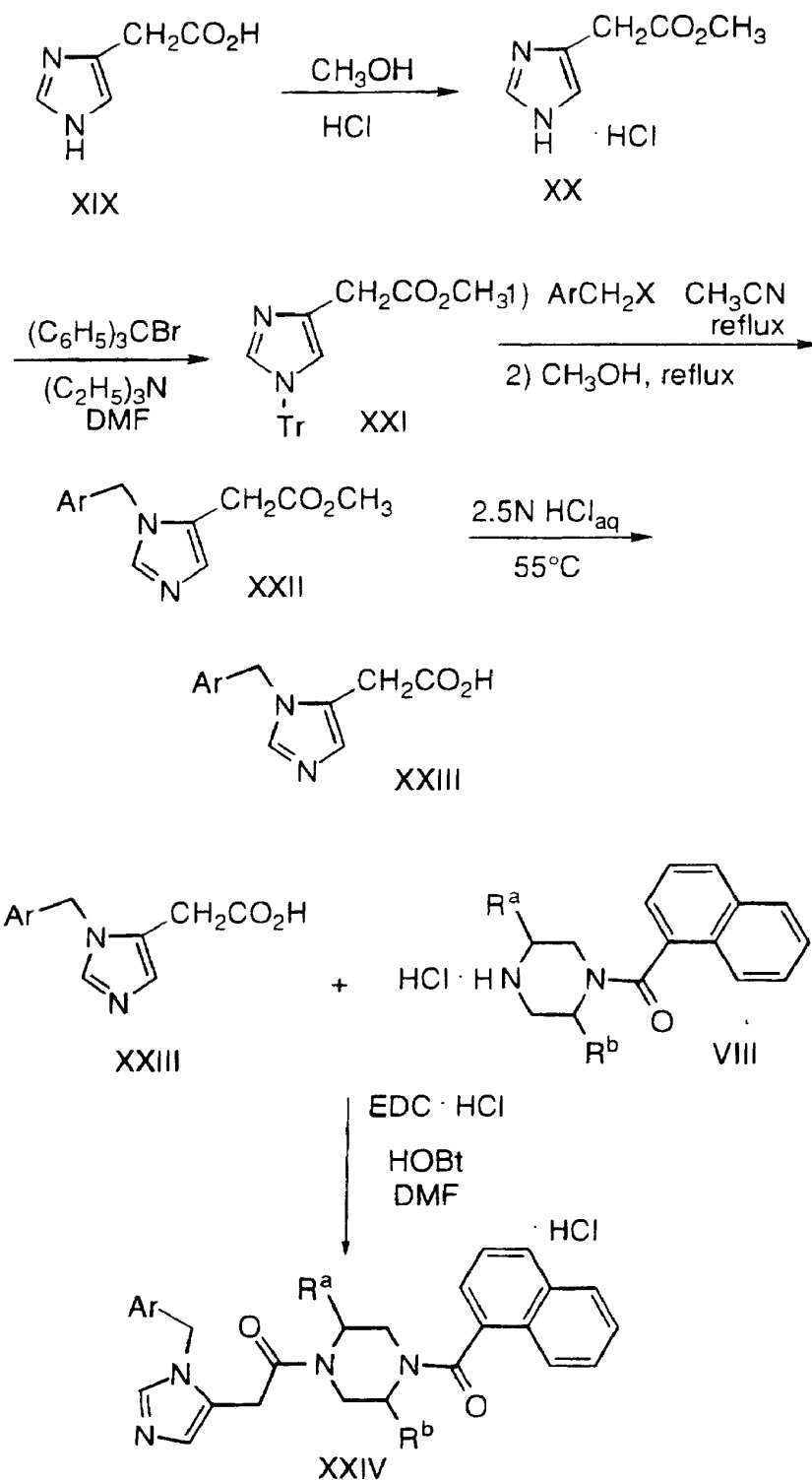
-135-

SCHEME 3 (Cont.)

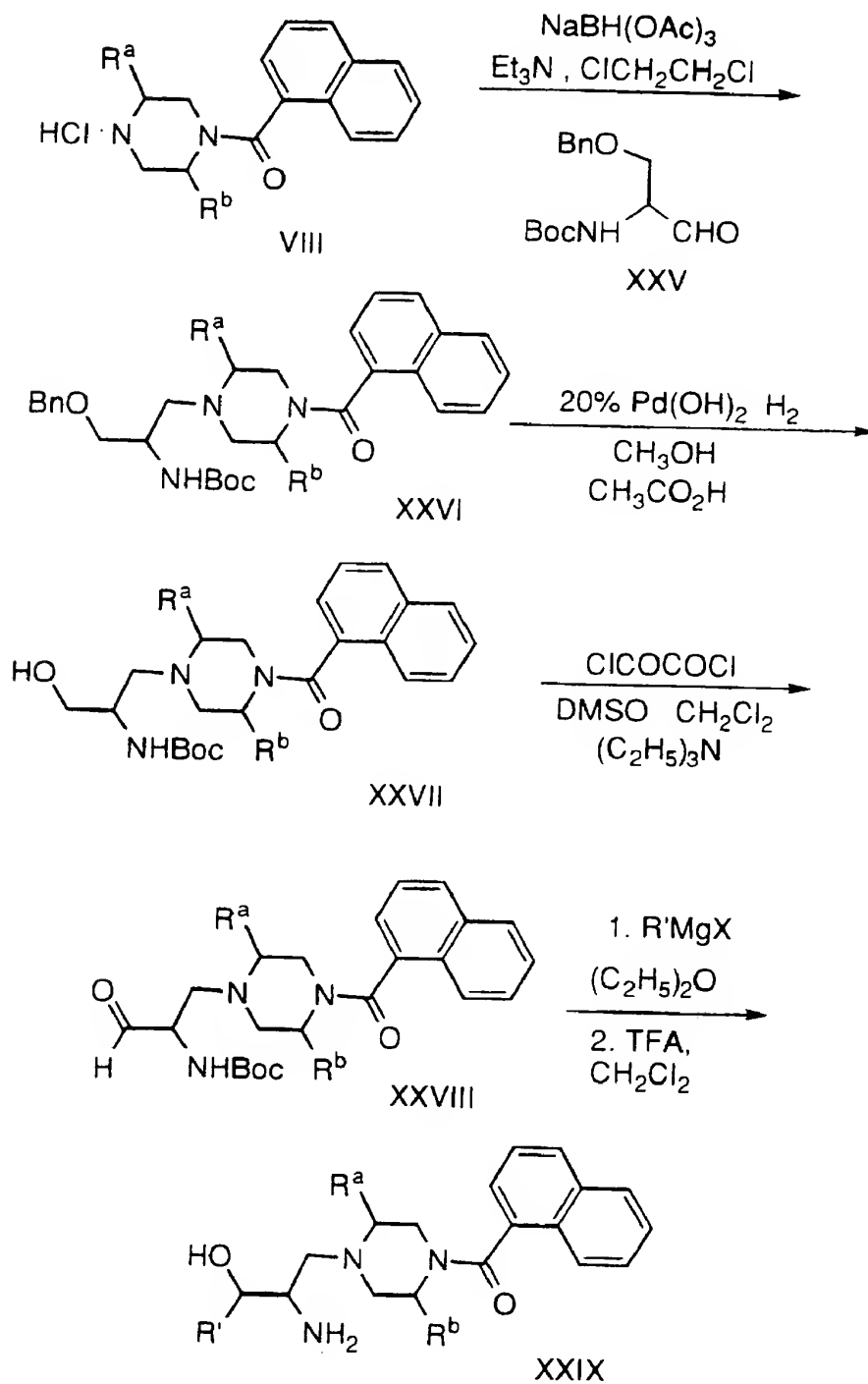
-136-

SCHEME 4

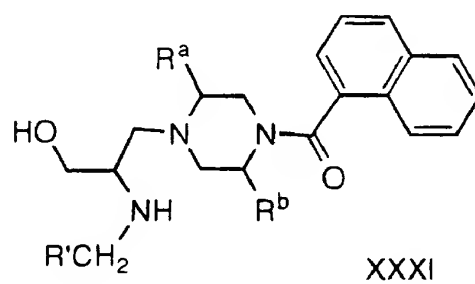
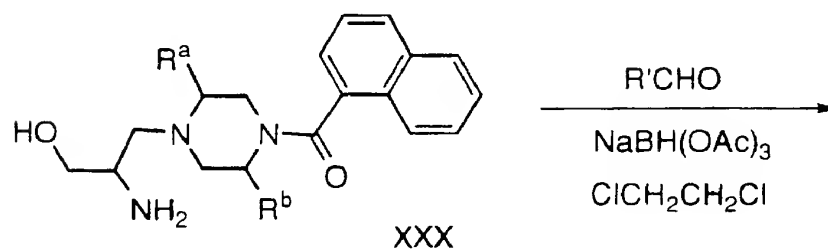
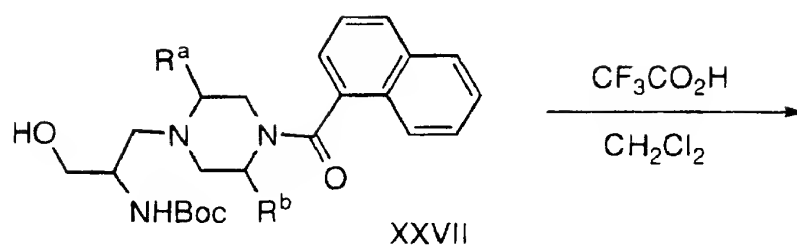
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SCHEME 5

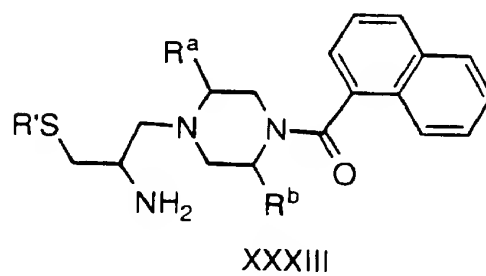
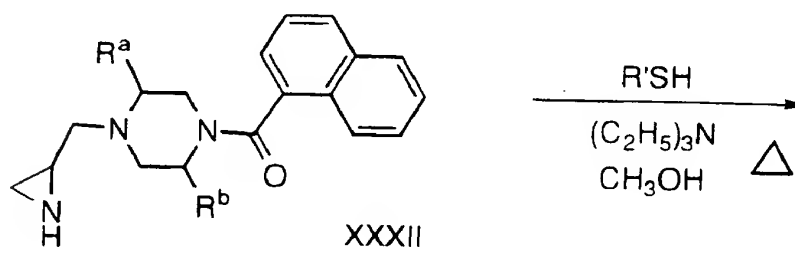
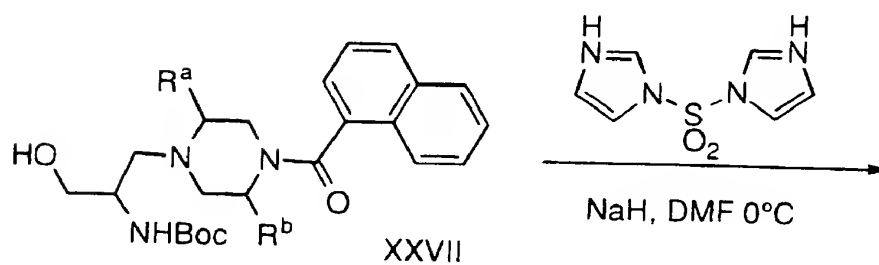
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SCHEME 6

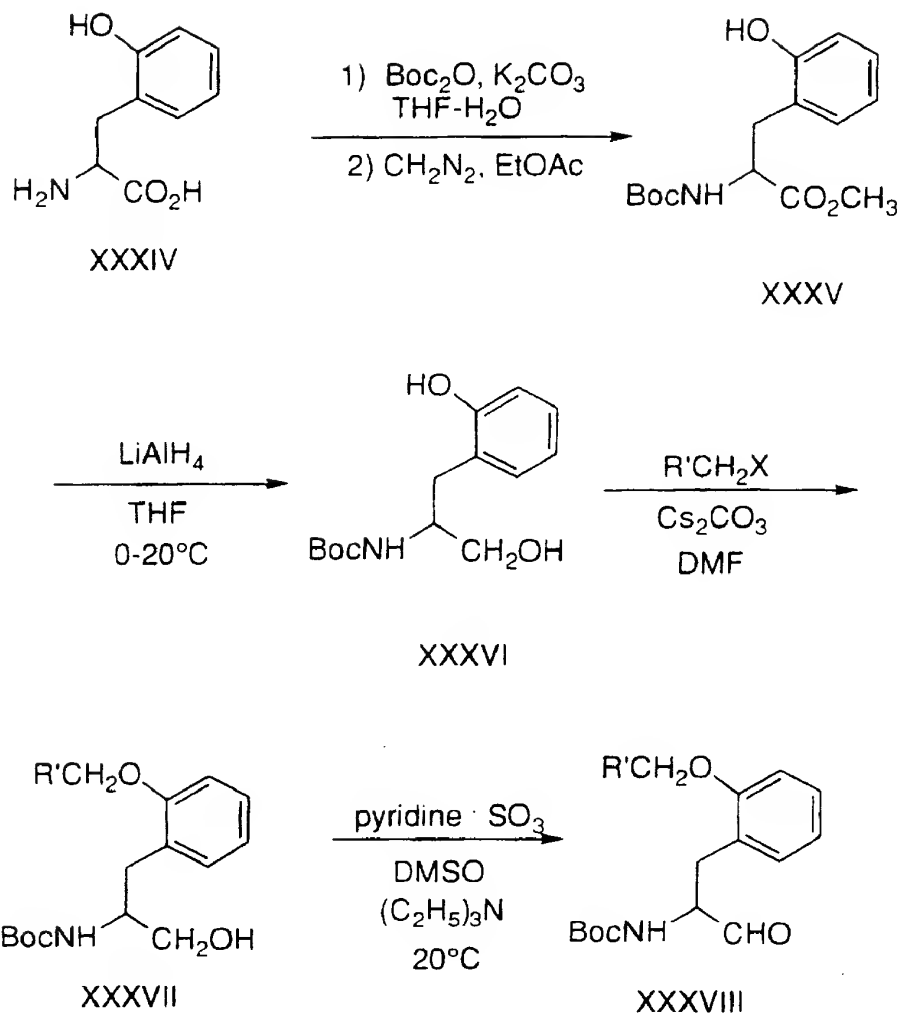
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SCHEME 7

-140-

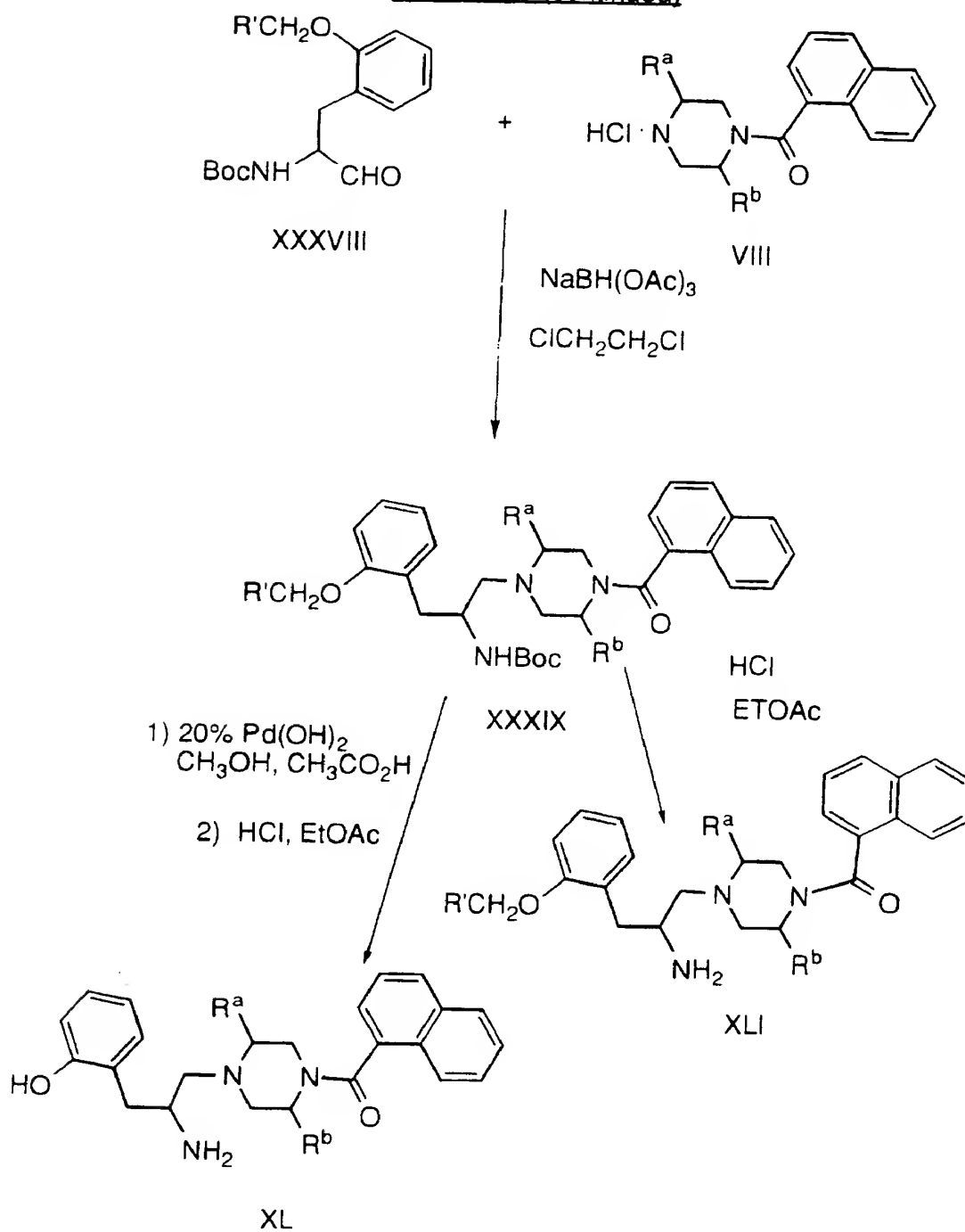
SCHEME 8

-141-

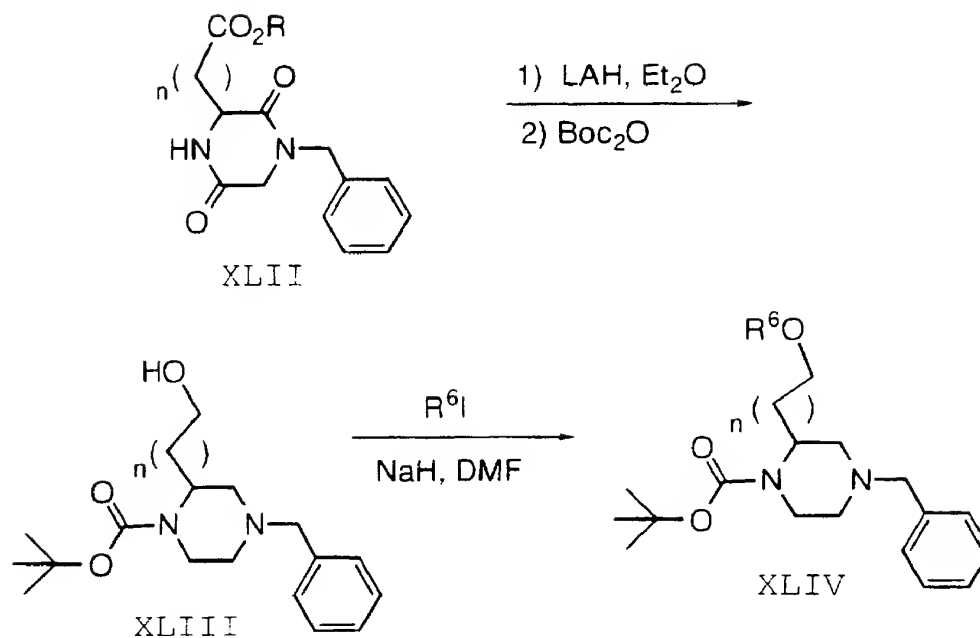
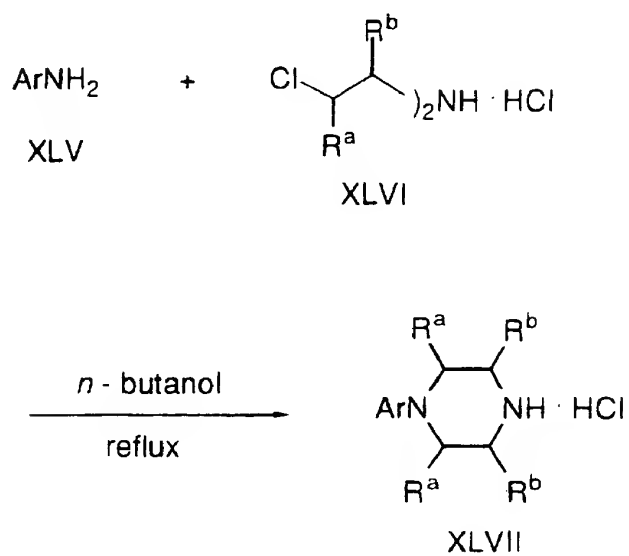
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-142-

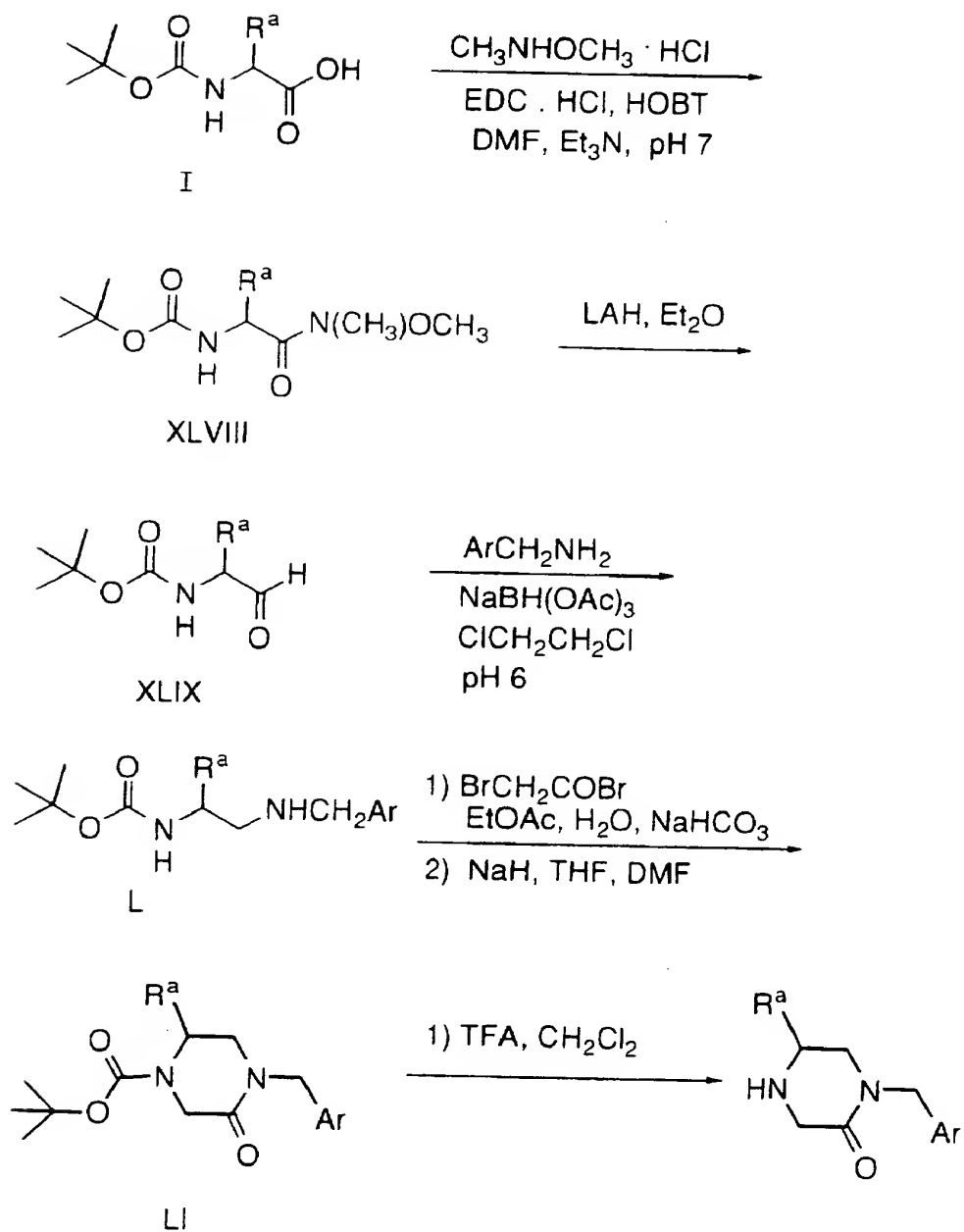
SCHEME 9 (continued)



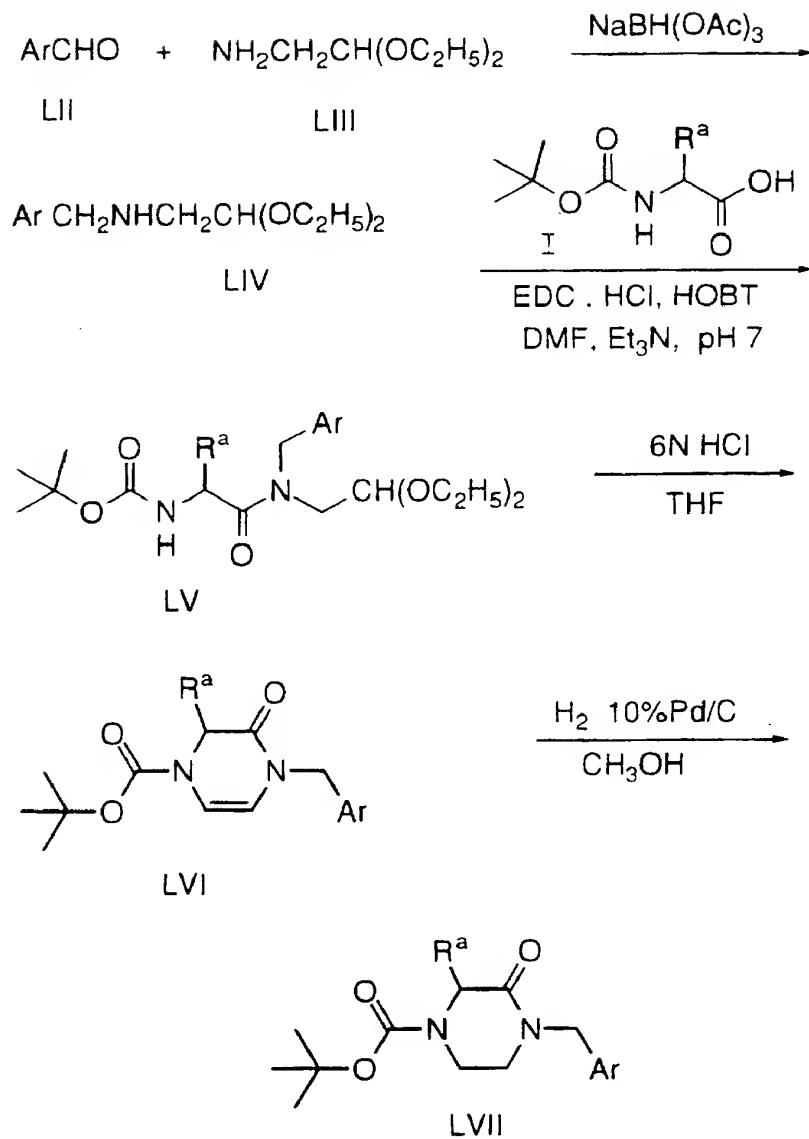
-143-

SCHEME 10SCHEME 11

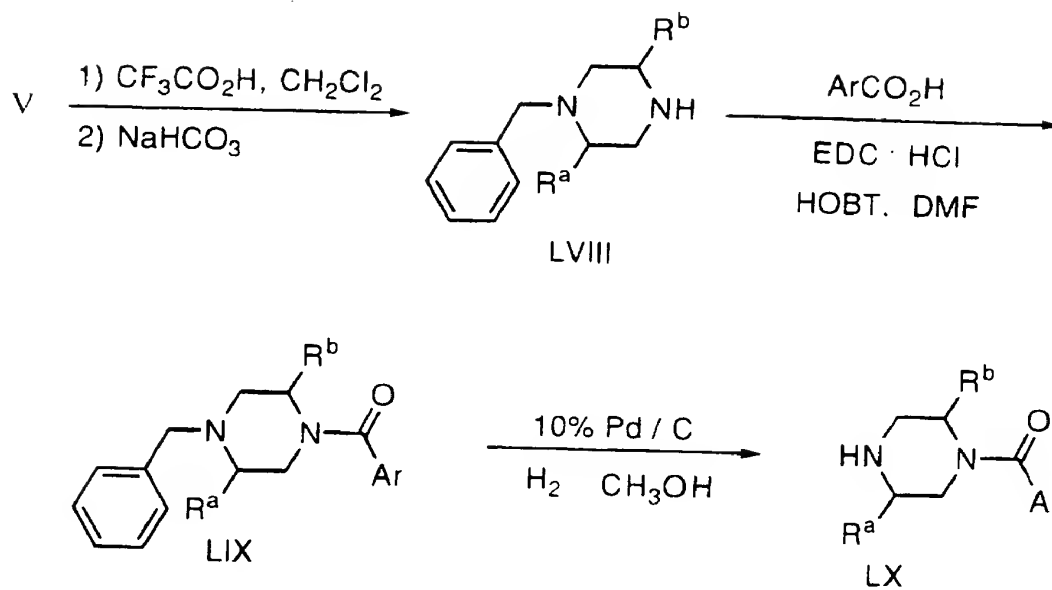
-144-

SCHEME 12

-145-

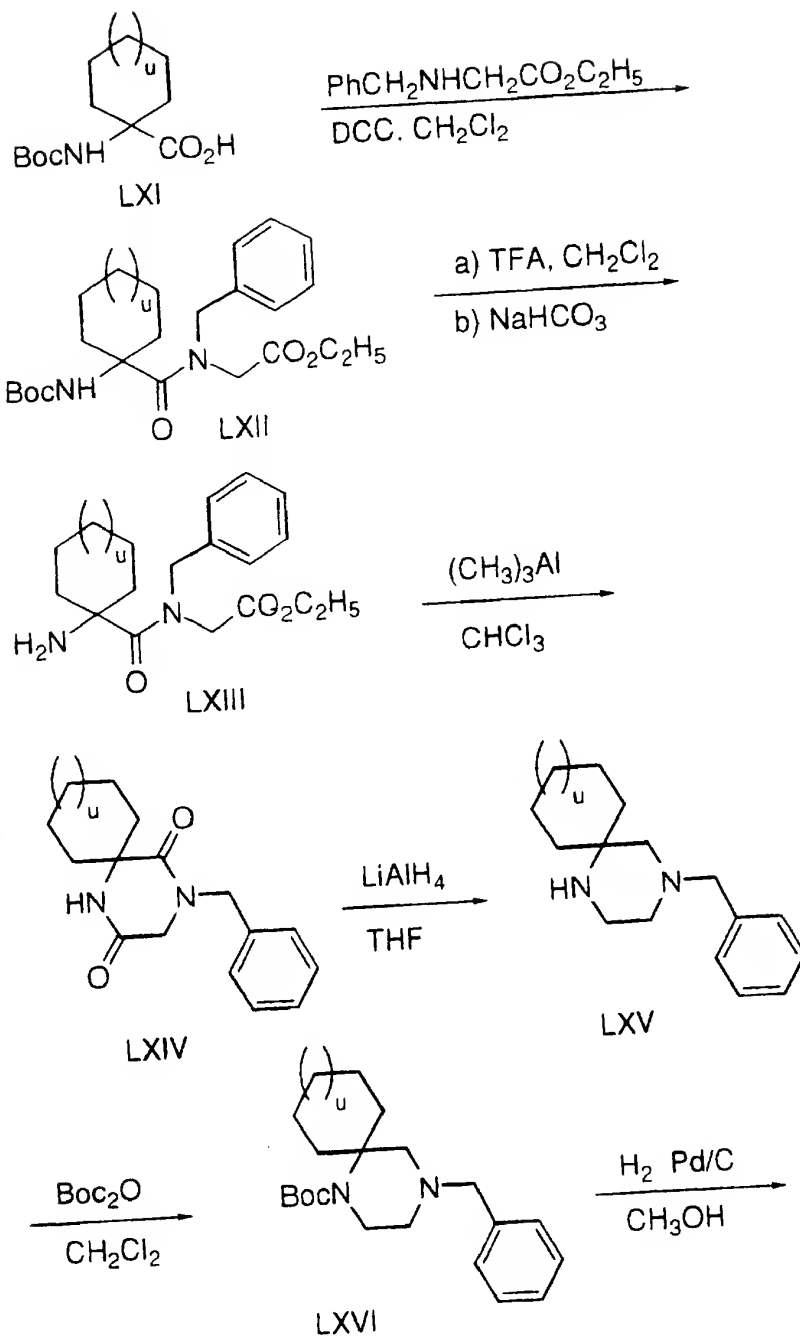
SCHEME 13

-146-

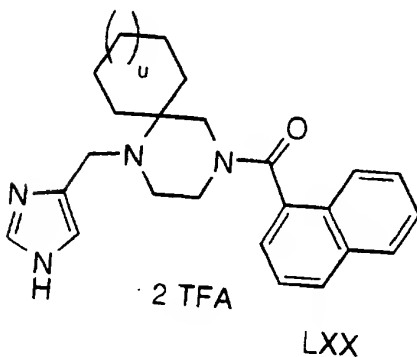
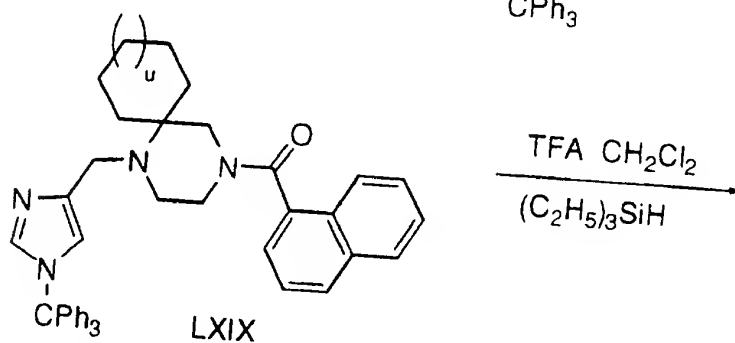
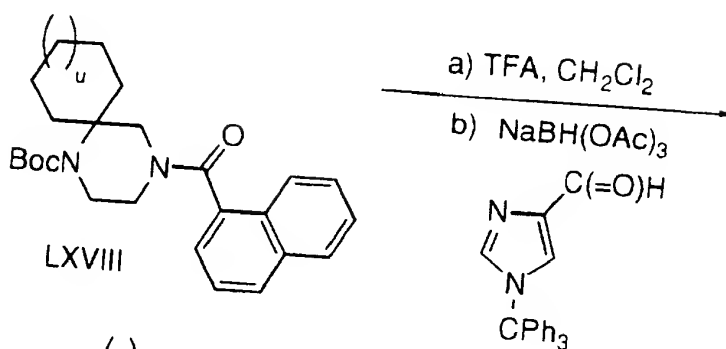
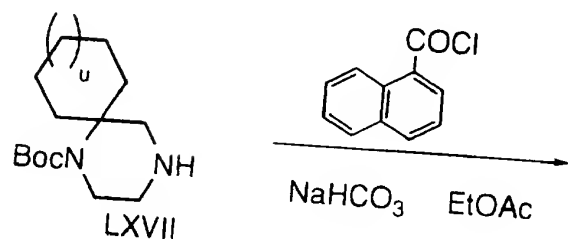
SCHEME 14

-147-

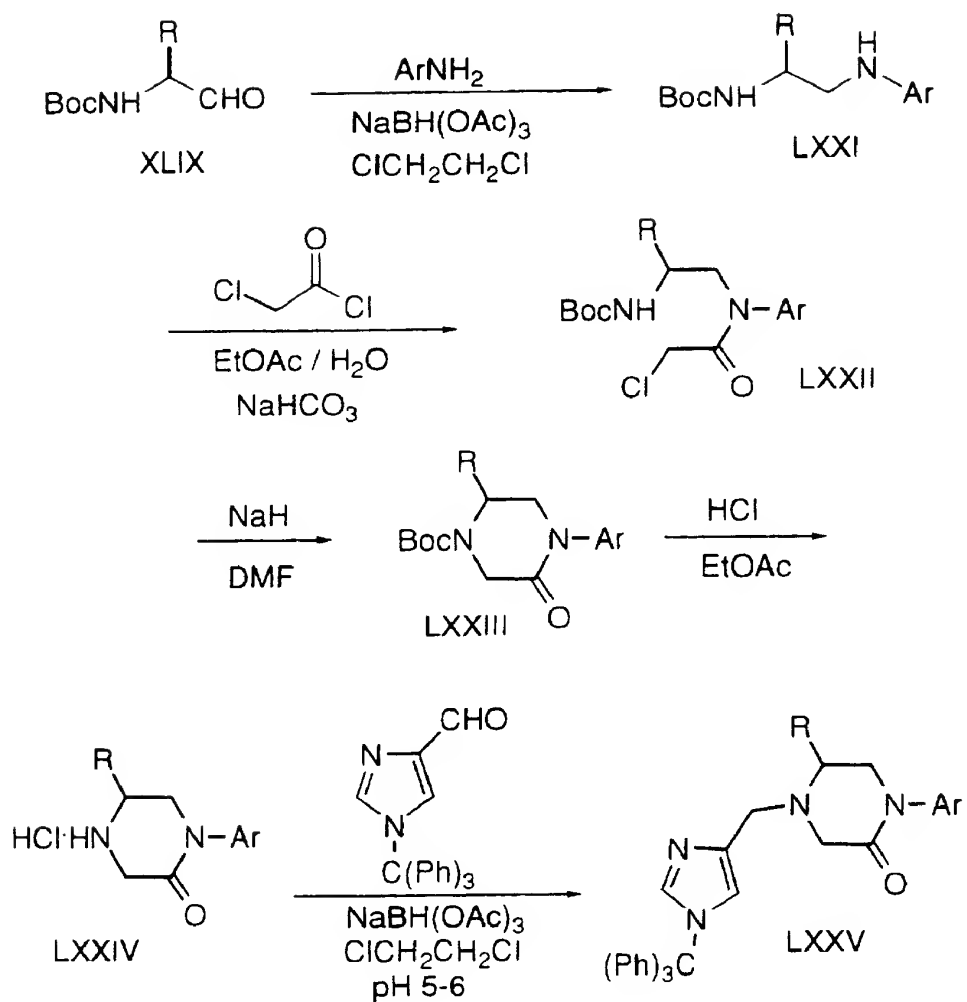
SCHEME 15



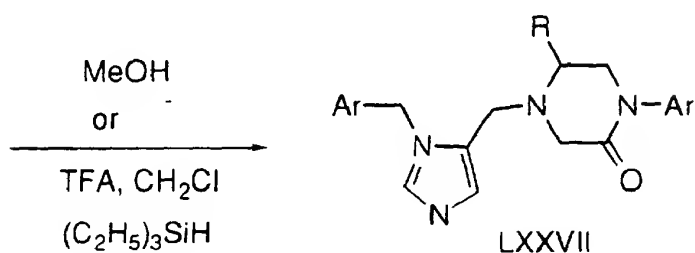
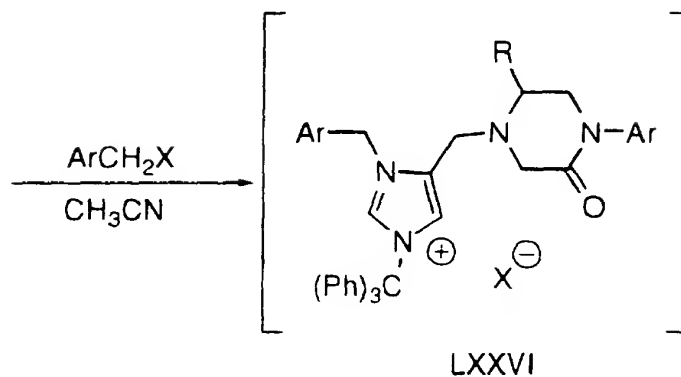
-148-

SCHEME 15 (continued)

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SCHEME 16

-150-

Scheme 6 (Continued)

5

The farnesyl transferase inhibitors can be synthesized in accordance with the general reaction schemes in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures. Some key bond-forming and peptide modifying reactions are:

Reaction A Amide bond formation and protecting group cleavage using standard solution or solid phase methodologies.

Reaction B Preparation of a reduced peptide subunit by reductive alkylation of an amine by an aldehyde using sodium cyanoborohydride or other reducing agents.

20

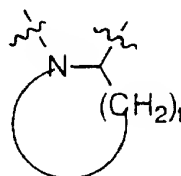
-151-

5 Reaction C Alkylation of a reduced peptide subunit with an alkyl or aralkyl halide or, alternatively, reductive alkylation of a reduced peptide subunit with an aldehyde using sodium cyanoborohydride or other reducing agents.

Reaction D Peptide bond formation and protecting group cleavage using standard solution or solid phase methodologies.

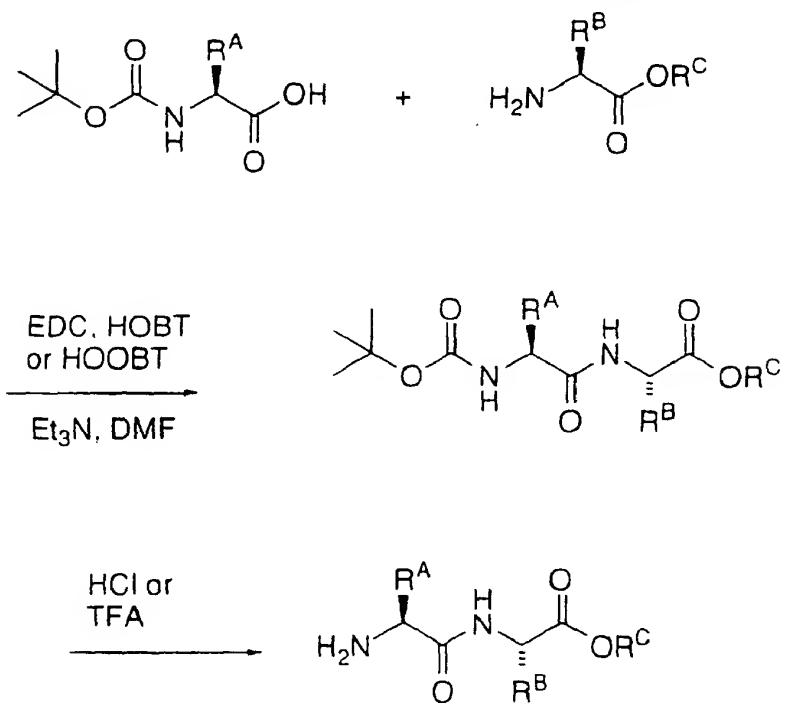
10 Reaction E Preparation of a reduced subunit by borane reduction of the amide moiety.

 Reaction Schemes A-E illustrate bond-forming and peptide modifying reactions incorporating acyclic peptide units. Such reactions are equally useful when the - NHC(R^A) - moiety of the reagents and
15 compounds illustrated is replaced with the following moiety:



 which can be substituted with R^{4a}, R^{4b}, R^{7a} and R^{7b} in accordance with structures (II-d) through (II-o). These reactions may be employed in a linear sequence to provide the compounds of the invention or they may
20 be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Reaction Schemes.

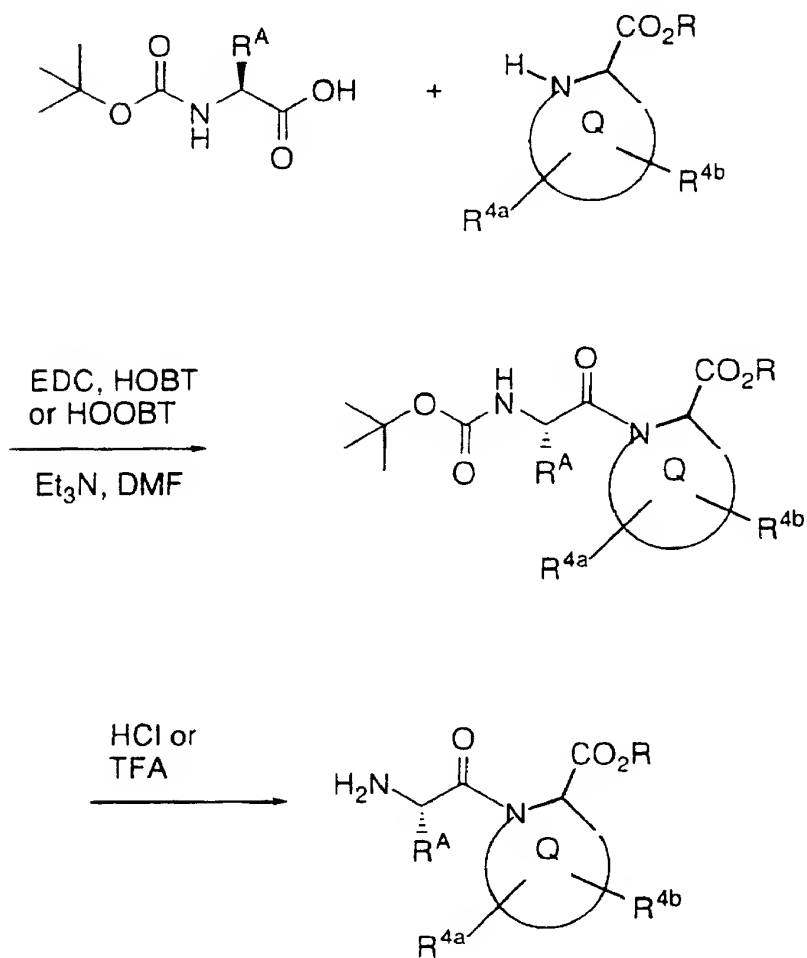
-152-

REACTION SCHEME AReaction A. Coupling of residues to form an amide bond

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ALTERNATIVE REACTION SCHEME A FORCOMPOUNDS (II-h) THROUGH (II-o)

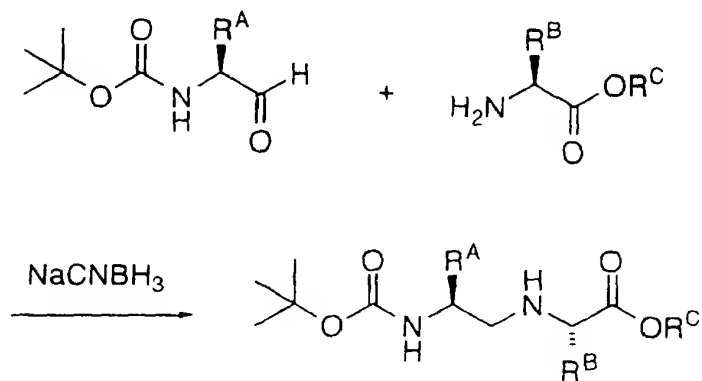
Coupling of residues to form an amide bond



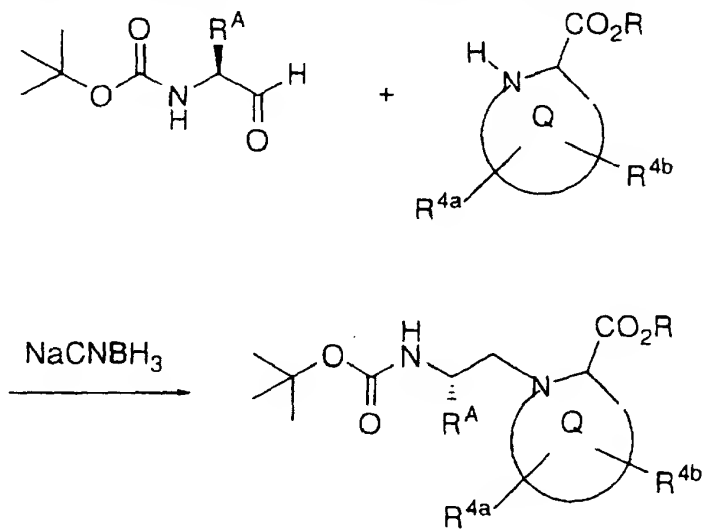
-154-

REACTION SCHEME B

Preparation of reduced peptide subunits by reductive alkylation

ALTERNATIVE REACTION SCHEME B FOR COMPOUNDS(II-h) THROUGH (II-o)

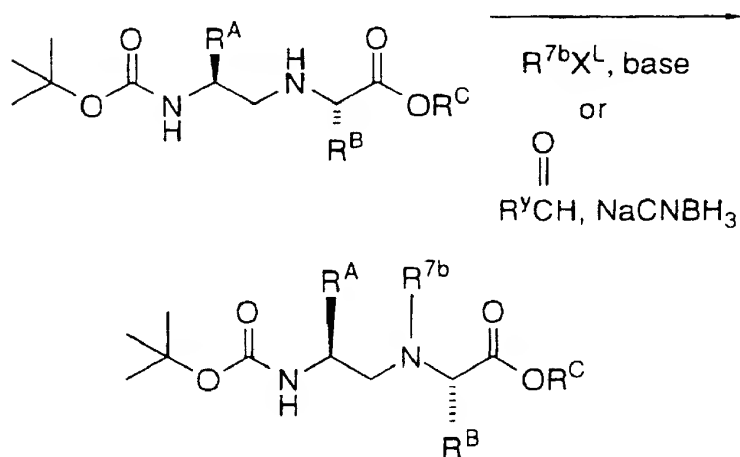
Preparation of reduced peptide subunits by reductive alkylation



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REACTION SCHEME C

Alkylation/reductive alkylation of reduced peptide subunits

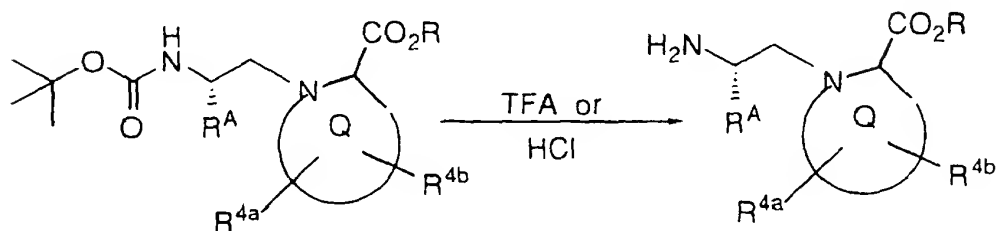


- where R^A and R^B are R^3 , R^4 , R^{5a} or R^{5b} as previously defined; R^C is R^6 as previously defined or a carboxylic acid protecting group; X^L is a leaving group, e.g., Br^- , I^- or MsO^- ; and R^Y is defined such that R^{7b} is generated by the reductive alkylation process.
- 5

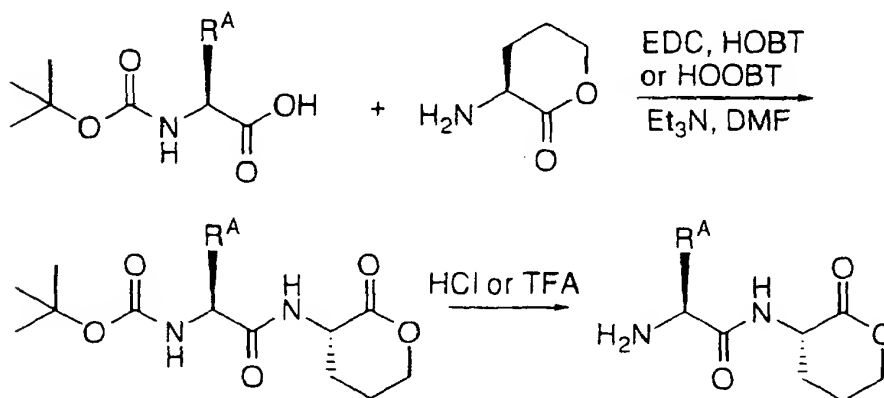
-156-

ALTERNATIVE REACTION SCHEME C for COMPOUNDS(II-h) THROUGH (II-o)

Deprotection of reduced peptide subunits

REACTION SCHEME D

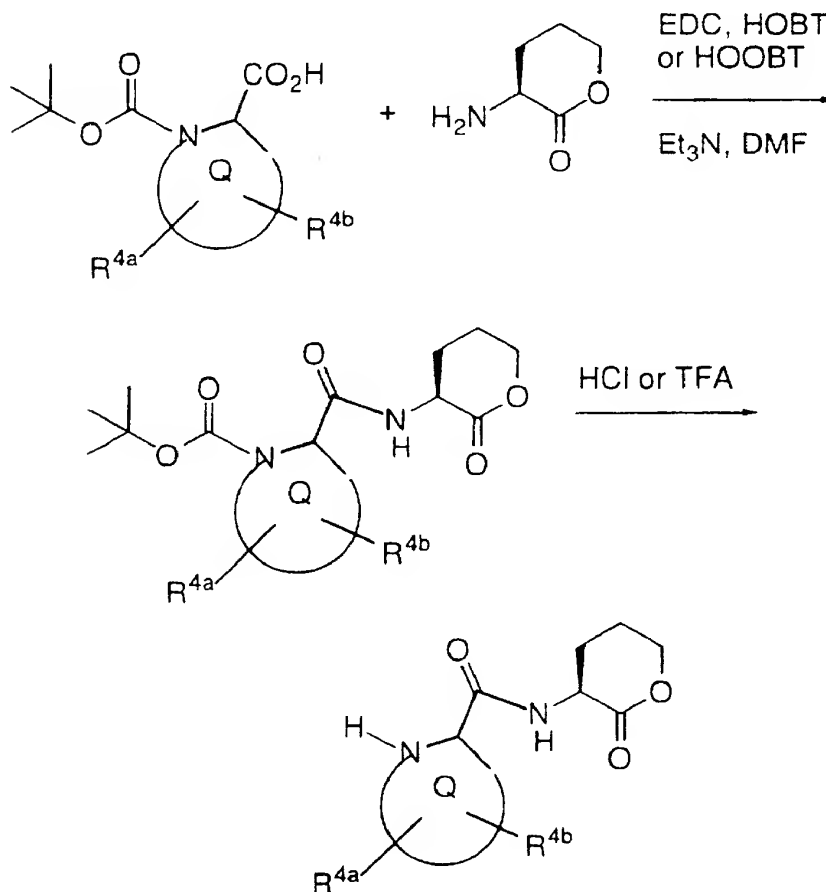
Coupling of residues to form an amide bond



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ALTERNATIVE REACTION SCHEME D FOR COMPOUNDS(II-h) THROUGH (II-o)

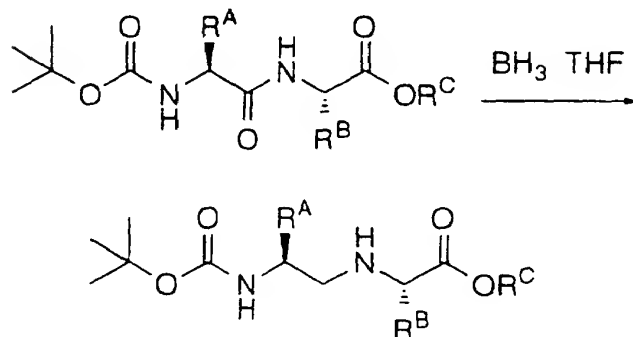
Coupling of residues to form an amide bond



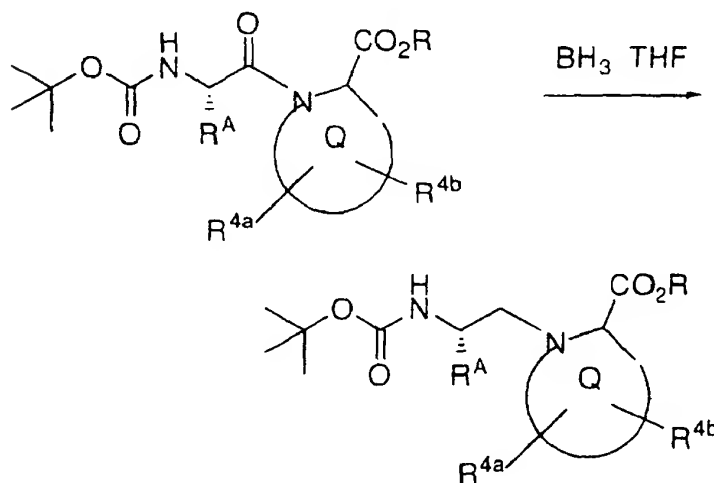
-158-

REACTION SCHEME E

Preparation of reduced dipeptides from peptides

ALTERNATIVE REACTION SCHEME E FOR COMPOUNDS(II-h) THROUGH (II-o)

Preparation of reduced dipeptides from peptides



All variables are as defined above.

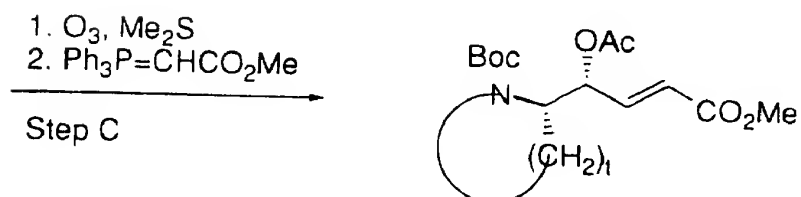
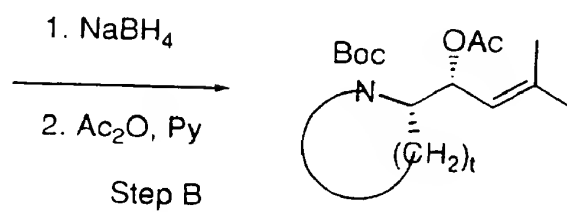
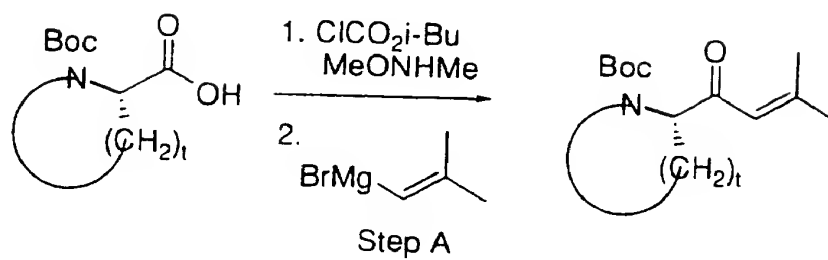
- 5 Certain compounds wherein X-Y is an ethylene or ethylene unit are prepared by employing the reaction sequences shown in Reaction Schemes F and G. Scheme F outlines the preparation of the alkene isosteres utilizing standard manipulations such as Weinreb amide formation, Grignard reaction, acetylation, ozonolysis, Wittig reaction,

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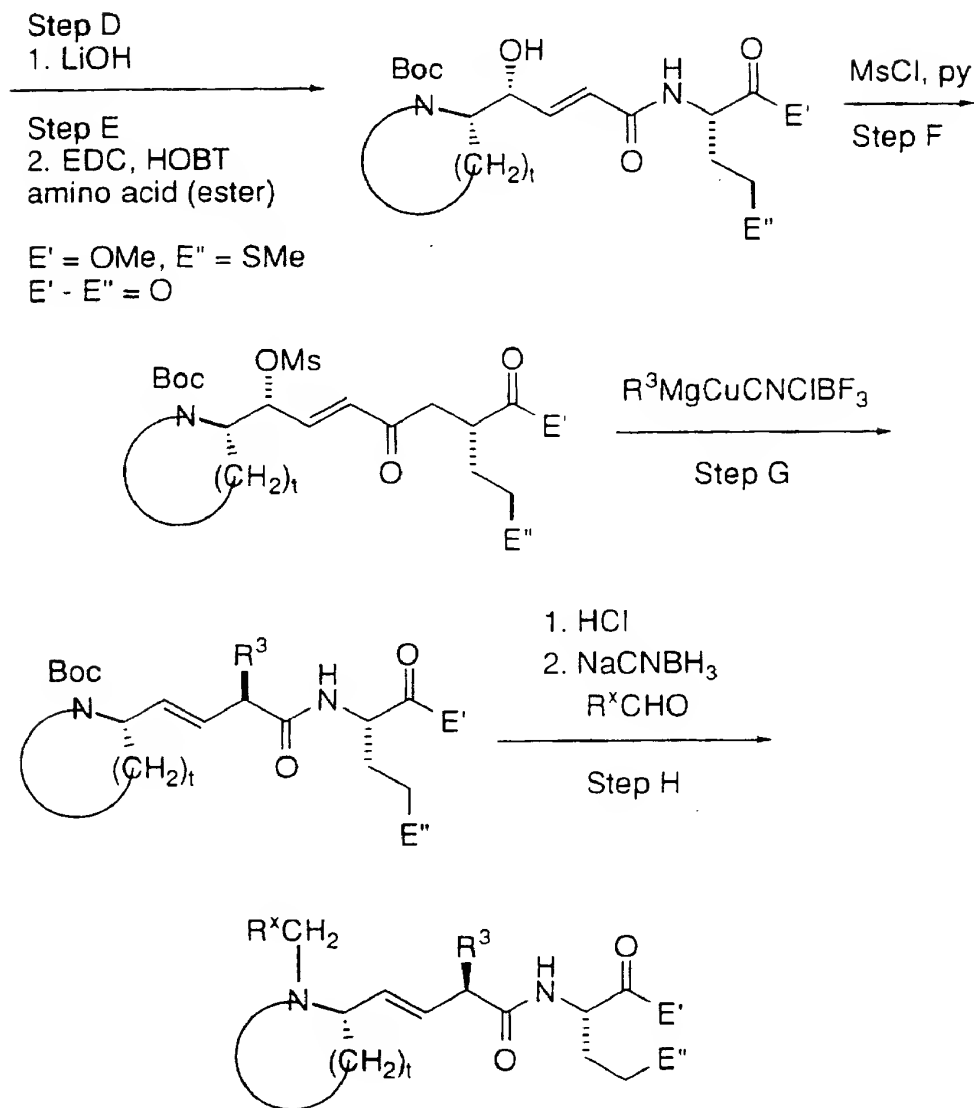
ester hydrolysis, peptide coupling reaction, mesylation, cleavage of peptide protecting groups, reductive alkylation, etc., as may be known in the literature or exemplified in the Experimental Procedure. For simplicity, substituents R^{2a} and R^{2b} on the cyclic amine moiety are not shown. It is, however, understood that the reactions illustrated are also applicable to appropriately substituted cyclic amine compounds. The key reactions are: stereoselective reduction of the Boc-aminoenone to the corresponding syn aminoalcohol (Scheme F, Step B, Part 1), and stereospecific boron trifluoride or zinc chloride activated organo-magnesium, organo-lithio, or organo-zinc copper(I) cyanide S_N2' displacement reaction (Scheme F, Step G). Through the use of optically pure N-Boc amino acids as starting material and these two key reactions, the stereochemistry of the final products is well defined. In Step H of Scheme F, the amino terminus sidechain, designated R^x is incorporated using coupling reaction A and R^xCOOH ; the alkylation reaction C using R^xCHO and a reducing agent; or alkylation reaction C using R^xCH_2XL . Such reactions as described in Step H are described in more detail in Reaction Schemes J-X hereinbelow.

The alkane analogs are prepared in a similar manner by including an additional catalytic hydrogenation step as outlined in Reaction Scheme G.

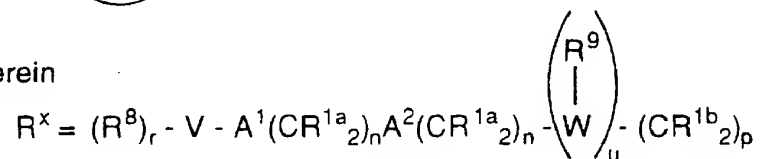
-160-

REACTION SCHEME F

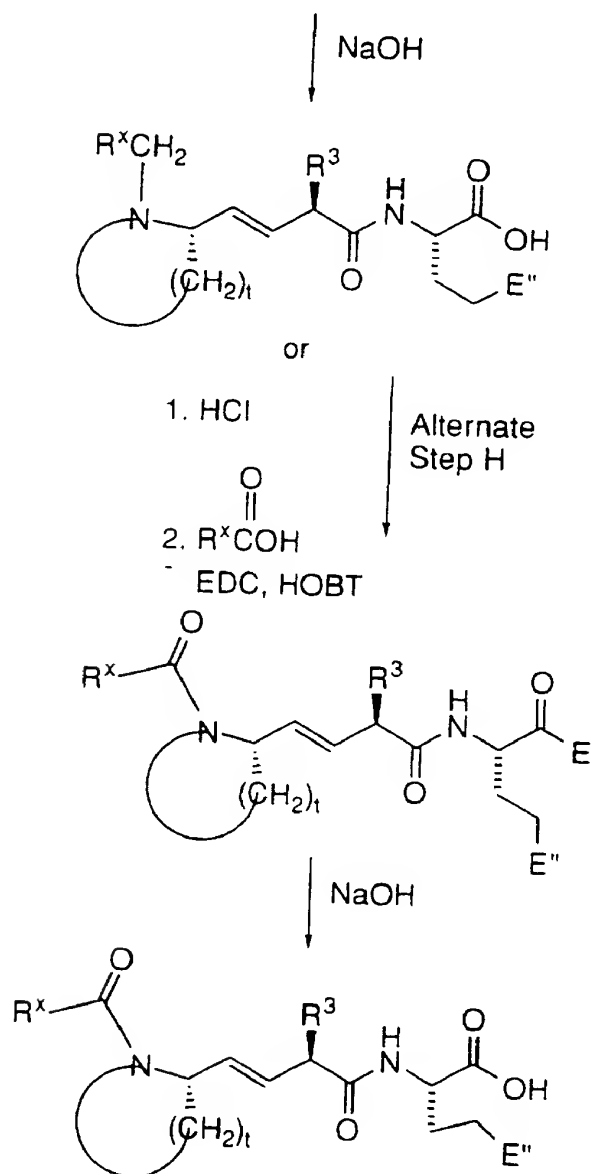
-161-

REACTION SCHEME F (CONT'D)

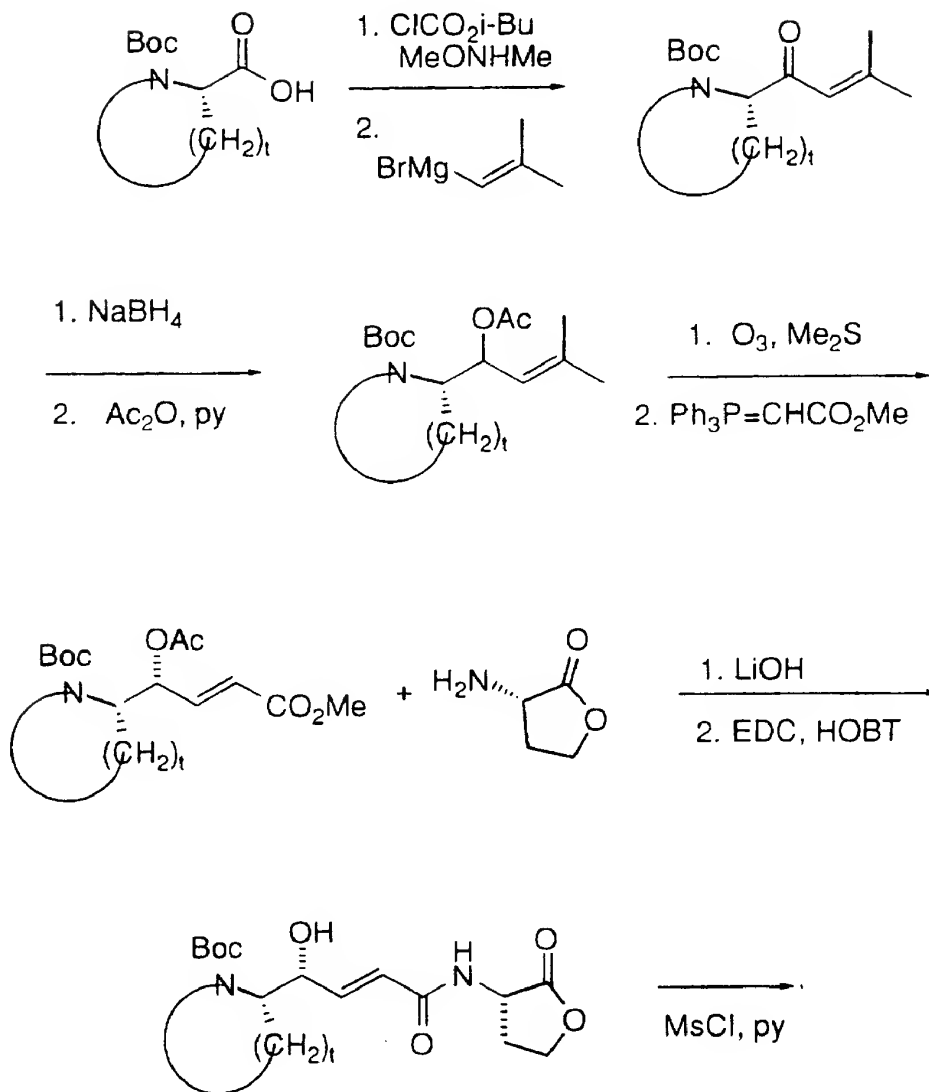
wherein



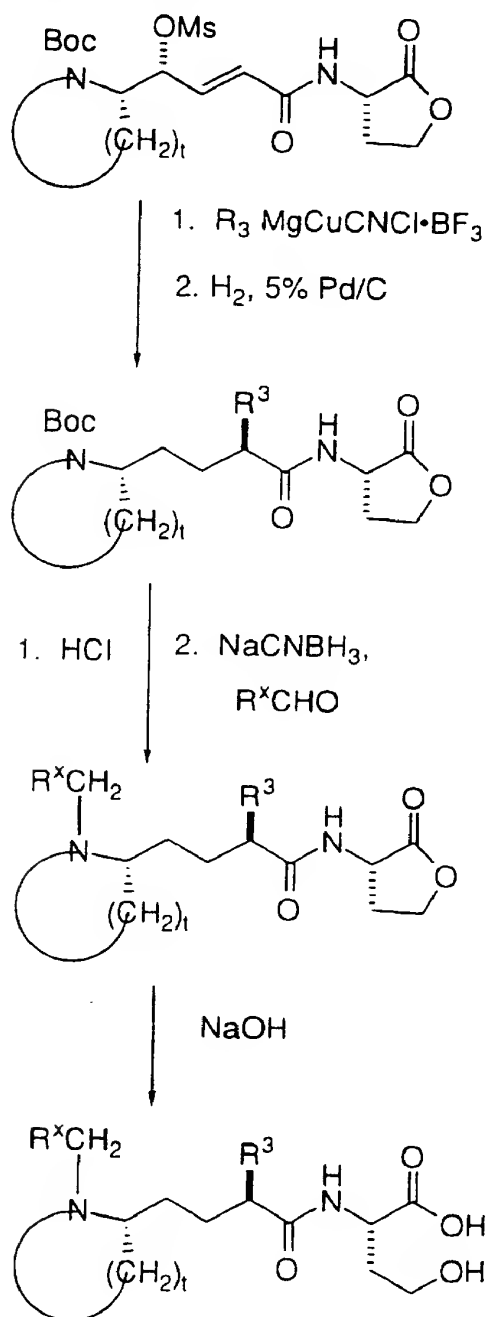
-162-

REACTION SCHEME F (CONT'D)

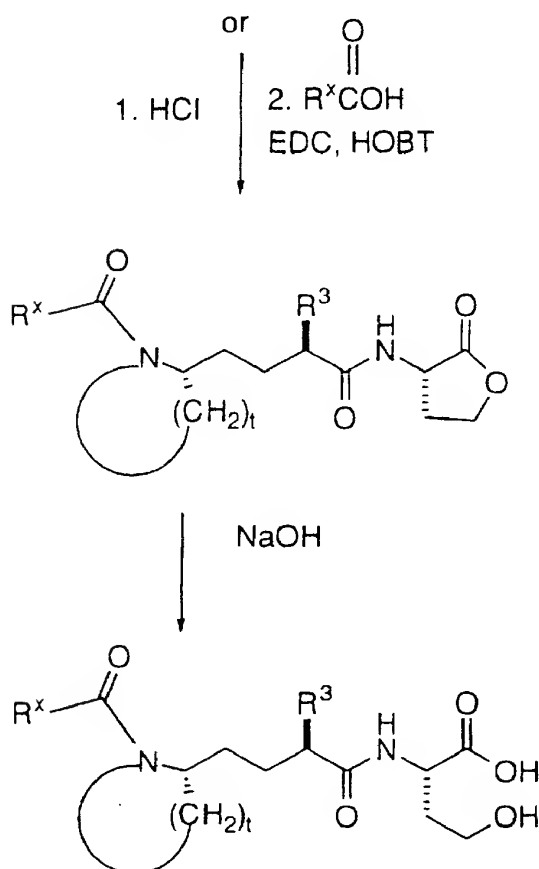
-163-

REACTION SCHEME G

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REACTION SCHEME G (CONT'D)

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REACTION SCHEME G (CONT'D)

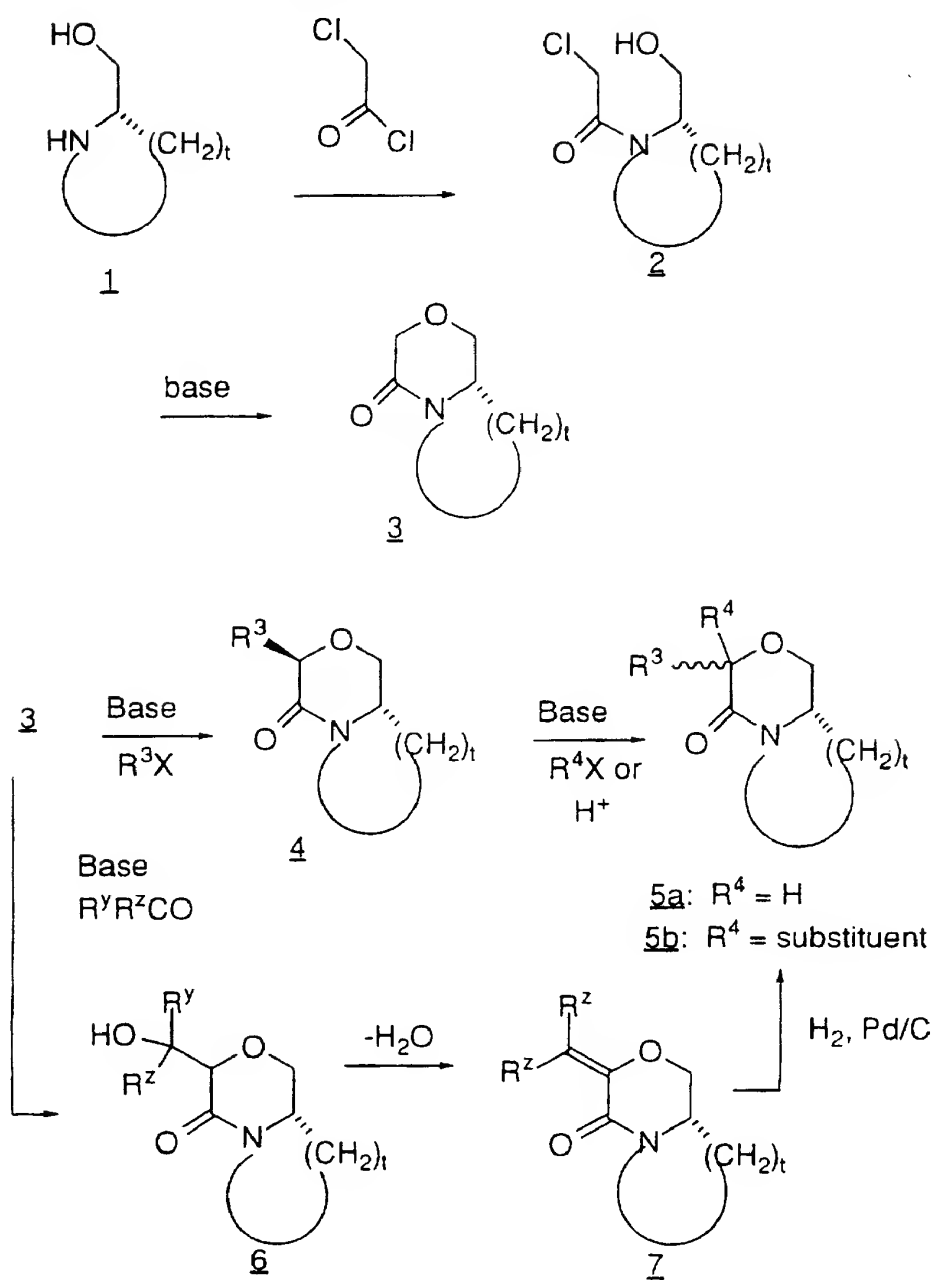
- The oxa isostere compounds of this invention are prepared according to the route outlined in Scheme H. An aminoalcohol 1 is
- 5 acylated with alpha-chloroacetyl chloride in the presence of trialkylamines to yield amide 2. Subsequent reaction of 2 with a deprotonation reagent (e.g., sodium hydride or potassium t-butoxide) in an ethereal solvent such as THF provides morpholinone 3. Alkylation of 3 with R³XL, where XL is a leaving group such as Br-, I- or Cl- in THF/DME
- 10 (1,2-dimethoxyethane) in the presence of a suitable base, preferably NaHMDS [sodium bis(trimethylsilyl)amide], affords 4, which is retreated with NaHMDS followed by either protonation or the addition of an alkyl halide R⁴X to give 5a or 5b, respectively, as a enantiomeric mixture. Alternatively, 5a can be prepared from 3 via an aldol

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condensation approach. Namely, deprotonation of 3 with NaHMDS followed by the addition of a carbonyl compound RYR^ZCO gives the adduct 6. Dehydration of 6 can be effected by mesylation and subsequent elimination catalyzed by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) or the direct treatment of 6 with phosphorus oxychloride in pyridine to give olefin 7. Then, catalytic hydrogenation of 7 yields 5a (wherein -CHRYR^Z constitutes R³). Direct hydrolysis of 5 with lithium hydrogen peroxide in aqueous THF, or aqueous HCl, produces acid 8a. Compound 8a is then derivatized with BOC-ON or BOC anhydride to give 8b. The peptide coupling of acid 8b with either an alpha-aminolactone (e.g., homoserine lactone, etc.) or the ester of an amino acid is carried out under the conditions exemplified in the previously described references to yield derivative 9. Treatment of 9 with gaseous hydrogen chloride gives 10, which undergoes further elaboration as described in Reaction Schemes J- hereinbelow.

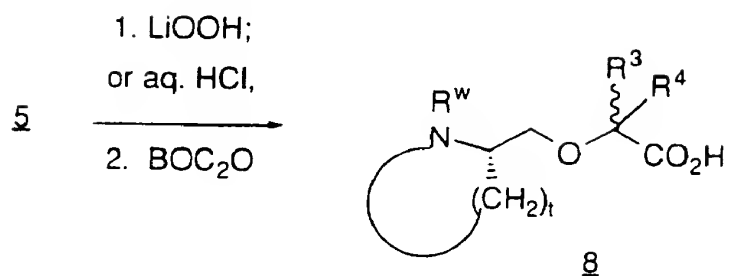
An alternative method for the preparation of the prolyl oxa isostere (compounds 23 and 24) is shown in Scheme H-1. Referring to Scheme H-1, the aminoalcohol 1 is protected with trifluoroacetic anhydride and the blocked compound 15 treated with diphenyl disulfide in the presence of tributylphosphine to provide the thioether 16. Chlorination of compound 16 provides compound 17 which can be reacted with the appropriate carboxylic acid alcohol in the presence of silver perchlorate and tin (II) chloride, to afford the mixed acetal 18. Removal of the phenylmercapto moiety with Raney nickel provides compound 19. Compound 19 is doubly deprotected, then selectively BOC protected to provide the acid 20, which undergoes the steps previously described for incorporating terminal amino acid. Still another alternative method for the preparation of the prolyl oxa isostere (compounds 23 and 24) is described in the literature [Ruth E. TenBrink, J. Org. Chem., **52**, 418-422 (1987)].

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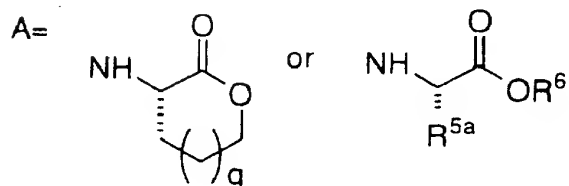
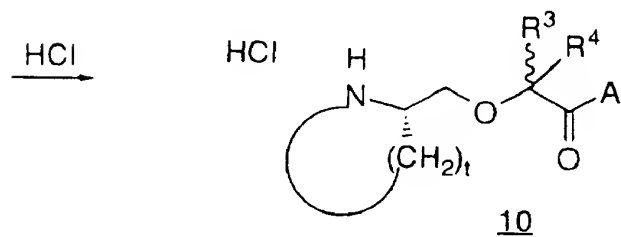
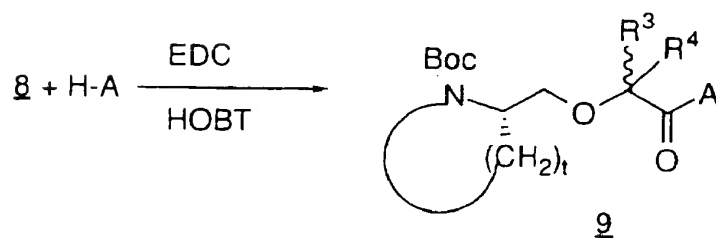
SCHEME H

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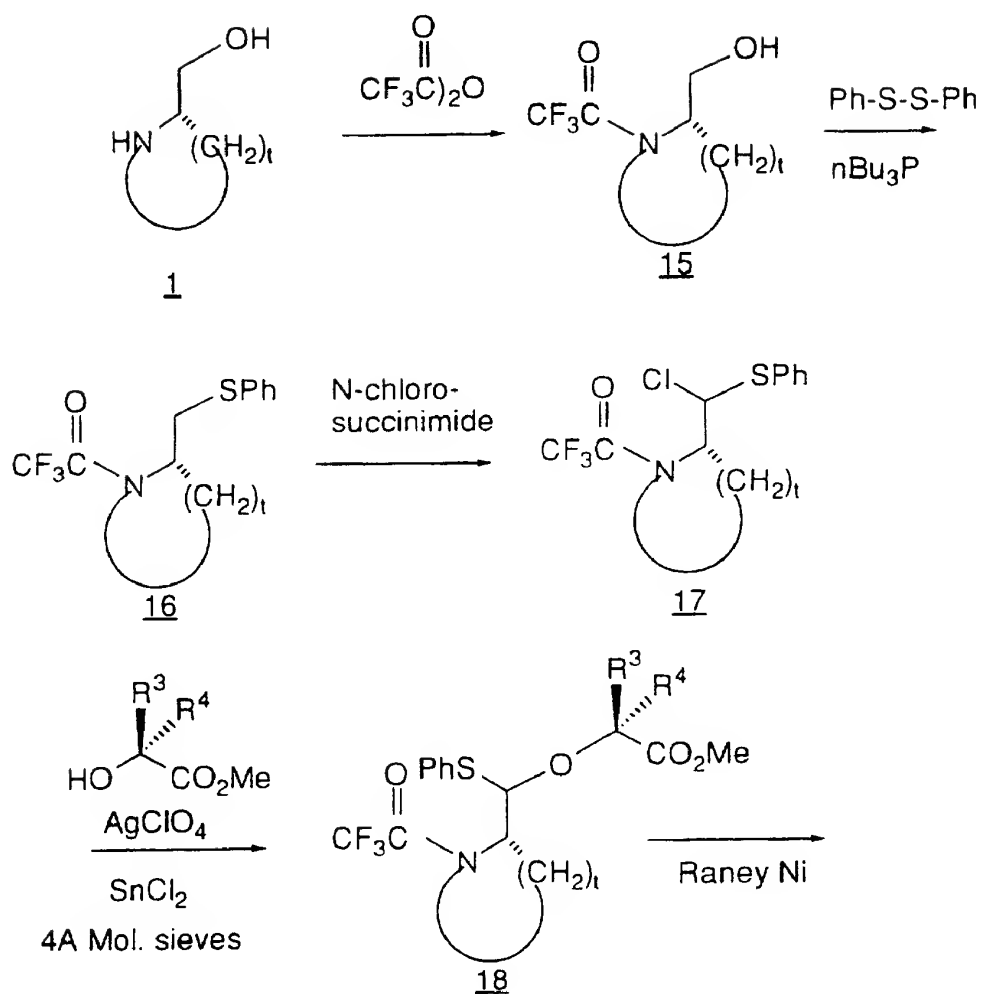
SCHEME H (CONT'D)



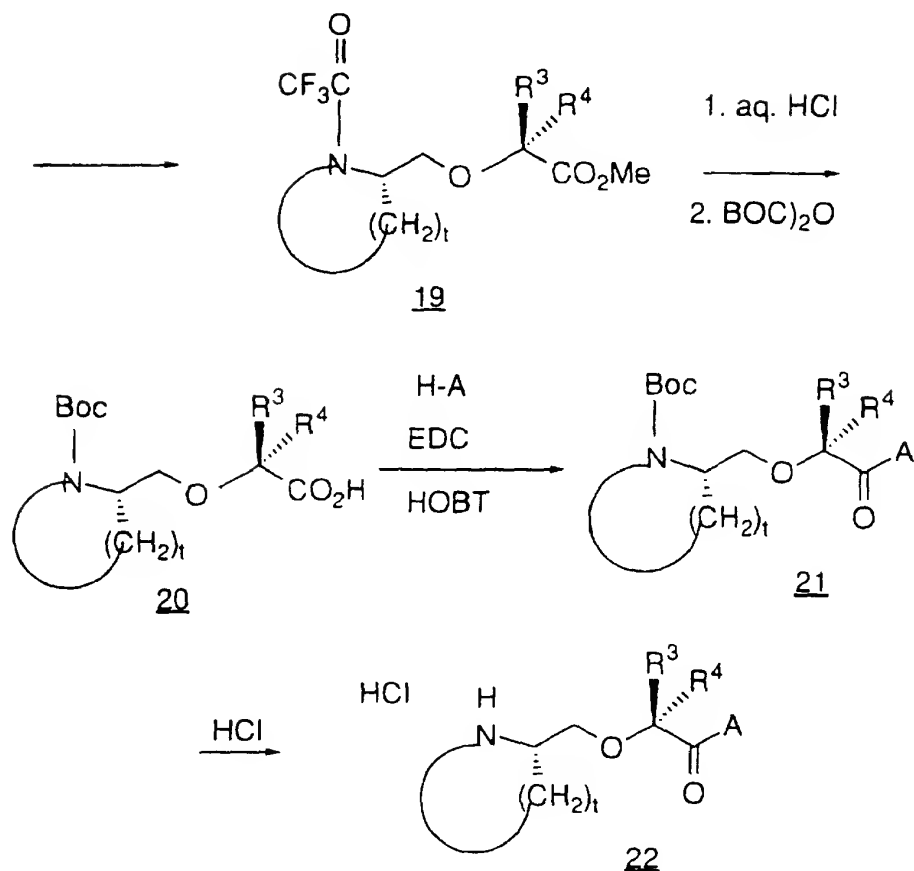
a, $R^w = \text{H}$
 b, $R^w = \text{BOC}$



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SCHEME H-1

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SCHEME H-1 (CONT'D)

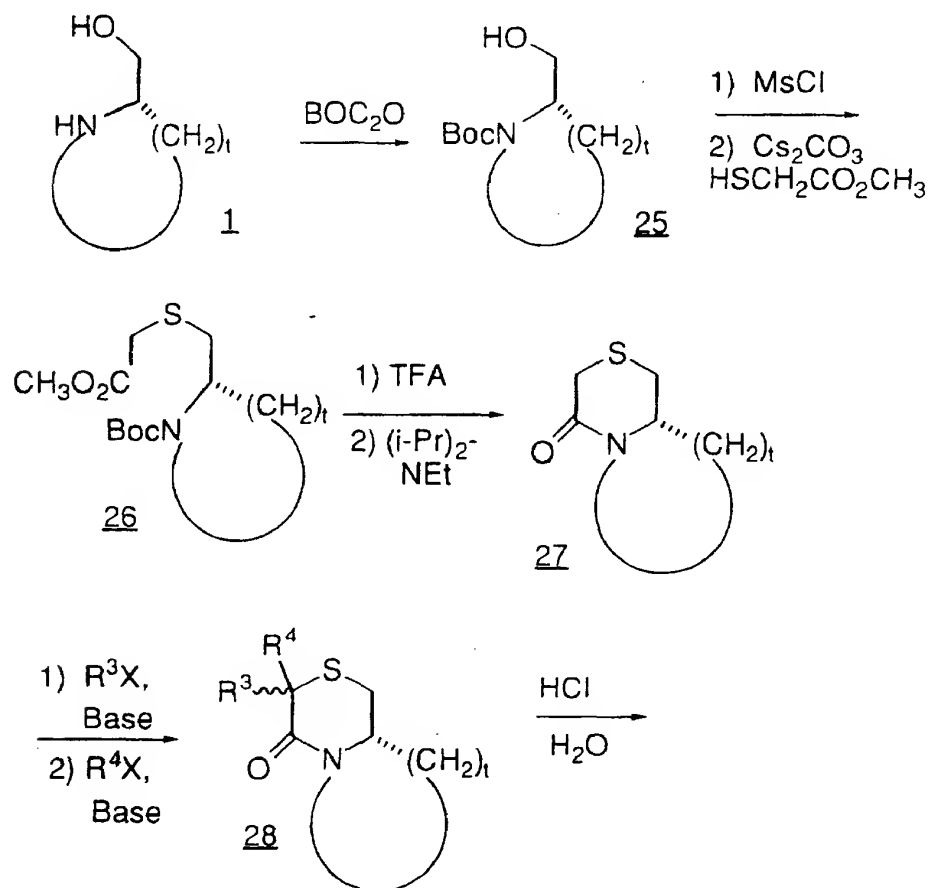
- The thia, oxothia and dioxothia isostere compounds of this invention are prepared in accordance to the route depicted in Scheme I.
- 5 Aminoalcohol 1 is derivatized with BOC_2O to give 25. Mesylation of 25 followed by reaction with methyl alpha-mercaptoacetate in the presence of cesium carbonate gives sulfide 26. Removal of the BOC group in 26 with TFA followed by neutralization with di-isopropyl-ethylamine leads to lactam 27. Sequential alkylation of 27 with the alkyl
- 10 halides R^3X and R^4X in THF/DME using NaHDMS as the deprotonation reagent produces 28. Hydrolysis of 28 in hydro-chloride to yield 29a, which is derivatized with Boc anhydride to yield 29b. The coupling of 29b with an alpha-aminolactone (e.g., homoserine lactone, etc.) or the ester of an amino acid is carried out under conventional conditions as

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exemplified in the previously described references to afford 30. Sulfide 30 is readily oxidized to sulfone 31 by the use of MCPBA (m-chloroperoxybenzoic acid). The N-BOC group of either 30 or 31 is readily removed by treatment with gaseous hydrogen chloride.

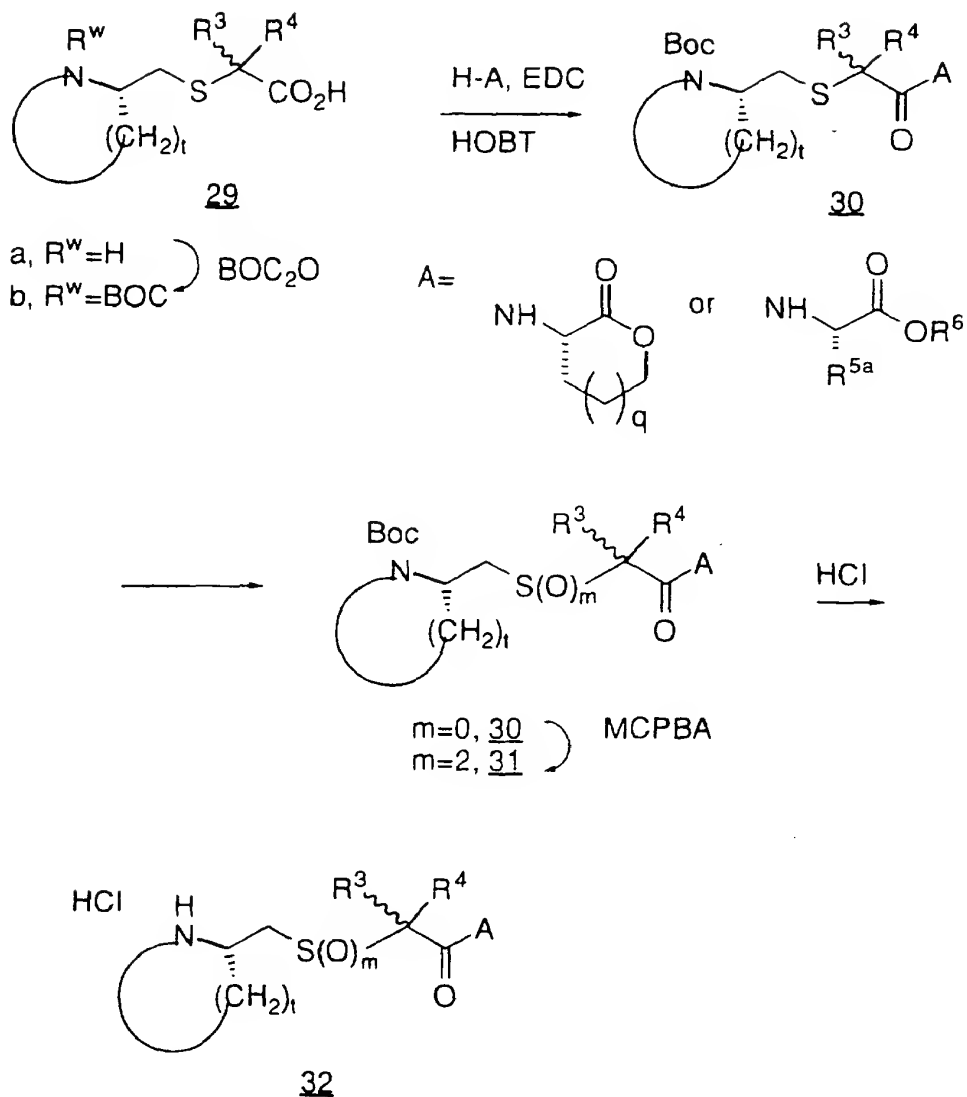
5

SCHEME I



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SCHEME I (Continued)



5

 $m = 0 \text{ or } 2$

Reaction Schemes J - R illustrate reactions wherein the non-sulfhydryl-containing moiety at the N-terminus of the compounds of the instant invention is attached to the fully elaborated cyclic amino peptide unit, prepared as described in Reaction Schemes A-I. It is understood

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that the reactions illustrated may also be performed on a simple cyclic amino acid, which may then be further elaborated utilizing reactions described in Reaction Schemes A- I to provide the instant compounds.

The intermediates whose synthesis are illustrated in

- 5 Reaction Schemes A-I can be reductively alkylated with a variety of aldehydes, such as V, as shown in Reaction Scheme J. The aldehydes can be prepared by standard procedures, such as that described by O. P. Goel, U. Krolls, M. Stier and S. Kesten in Organic Syntheses, 1988, 67, 69-75, from the appropriate amino acid (Reaction Scheme F).
- 10 The reductive alkylation can be accomplished at pH 5-7 with a variety of reducing agents, such as sodium triacetoxyborohydride or sodium cyanoborohydride in a solvent such as dichloroethane, methanol or dimethylformamide. The product VI can be deprotected with trifluoroacetic acid in methylene chloride to give the final compounds
- 15 VII. The final product VII is isolated in the salt form, for example, as a trifluoroacetate, hydrochloride or acetate salt, among others. The product diamine VII can further be selectively protected to obtain VIII, which can subsequently be reductively alkylated with a second aldehyde to obtain IX. Removal of the protecting group, and
- 20 conversion to cyclized products such as the dihydroimidazole XI can be accomplished by literature procedures.

- Alternatively, the protected cyclic aminopeptidyl intermediate can be reductively alkylated with other aldehydes such as 1-trityl-4-carboxaldehyde or 1-trityl-4-imidazolylacetaldehyde, to give
- 25 products such as XII (Reaction Scheme K). The trityl protecting group can be removed from XII to give XIII, or alternatively, XII can first be treated with an alkyl halide then subsequently deprotected to give the alkylated imidazole XIV. Alternatively, the dipeptidyl analog intermediate can be acylated or sulfonylated by standard techniques.

- 30 The imidazole acetic acid XV can be converted to the protected acetate XVII by standard procedures, and XVII can be first reacted with an alkyl halide, then treated with refluxing methanol to provide the regiospecifically alkylated imidazole acetic acid ester XVIII. Hydrolysis and reaction with the protected dipeptidyl analog

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intermediate in the presence of condensing reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) leads to acylated products such as XIX.

If the protected dipeptidyl analog intermediate is
5 reductively alkylated with an aldehyde which also has a protected hydroxyl group, such as XX in Reaction Scheme N, the protecting groups can be subsequently removed to unmask the hydroxyl group (Reaction Schemes N, P). The alcohol can be oxidized under standard conditions to *e.g.* an aldehyde, which can then be reacted with a variety
10 of organometallic reagents such as Grignard reagents, to obtain secondary alcohols such as XXIV. In addition, the fully deprotected amino alcohol XXV can be reductively alkylated (under conditions described previously) with a variety of aldehydes to obtain secondary amines, such as XXVI (Reaction Scheme P), or tertiary amines.

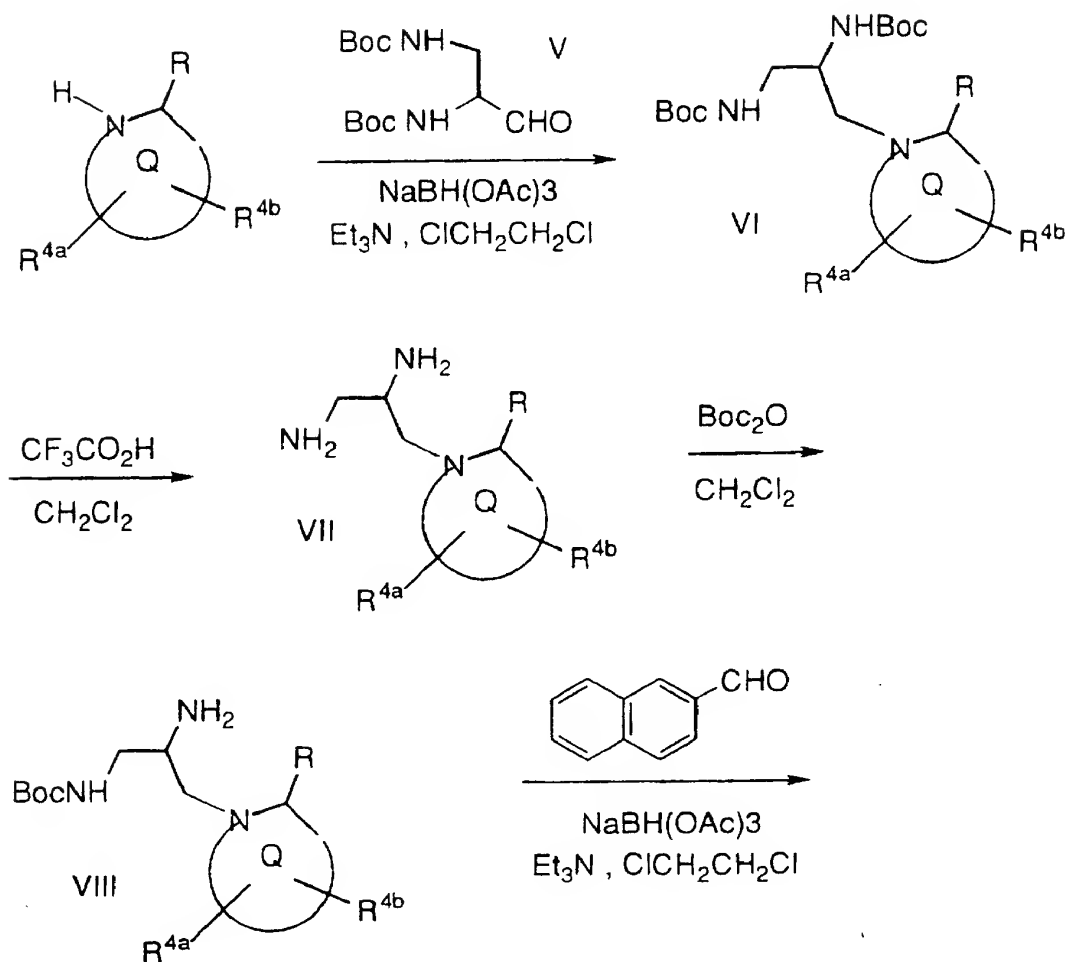
15 The Boc protected amino alcohol XXII can also be utilized to synthesize 2-aziridinylmethylpiperazines such as XXVII (Reaction Scheme Q). Treating XXII with 1,1'-sulfonyldiimidazole and sodium hydride in a solvent such as dimethylformamide led to the formation of aziridine XXVII. The aziridine may be reacted in the presence of a
20 nucleophile, such as a thiol, in the presence of base to yield the ring-opened product XXVIII.

In addition, the protected dipeptidyl analog intermediate can be reacted with aldehydes derived from amino acids such as O-alkylated tyrosines, according to standard procedures, to obtain
25 compounds such as XXXIV, as shown in Reaction Scheme R. When R' is an aryl group, XXXIV can first be hydrogenated to unmask the phenol, and the amine group deprotected with acid to produce XXXV. Alternatively, the amine protecting group in XXXIV can be removed, and O-alkylated phenolic amines such as XXXVI produced.

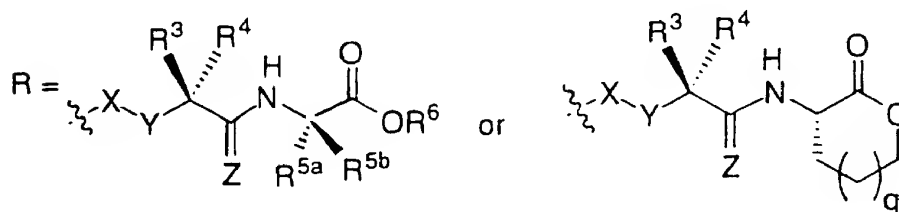
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-175-

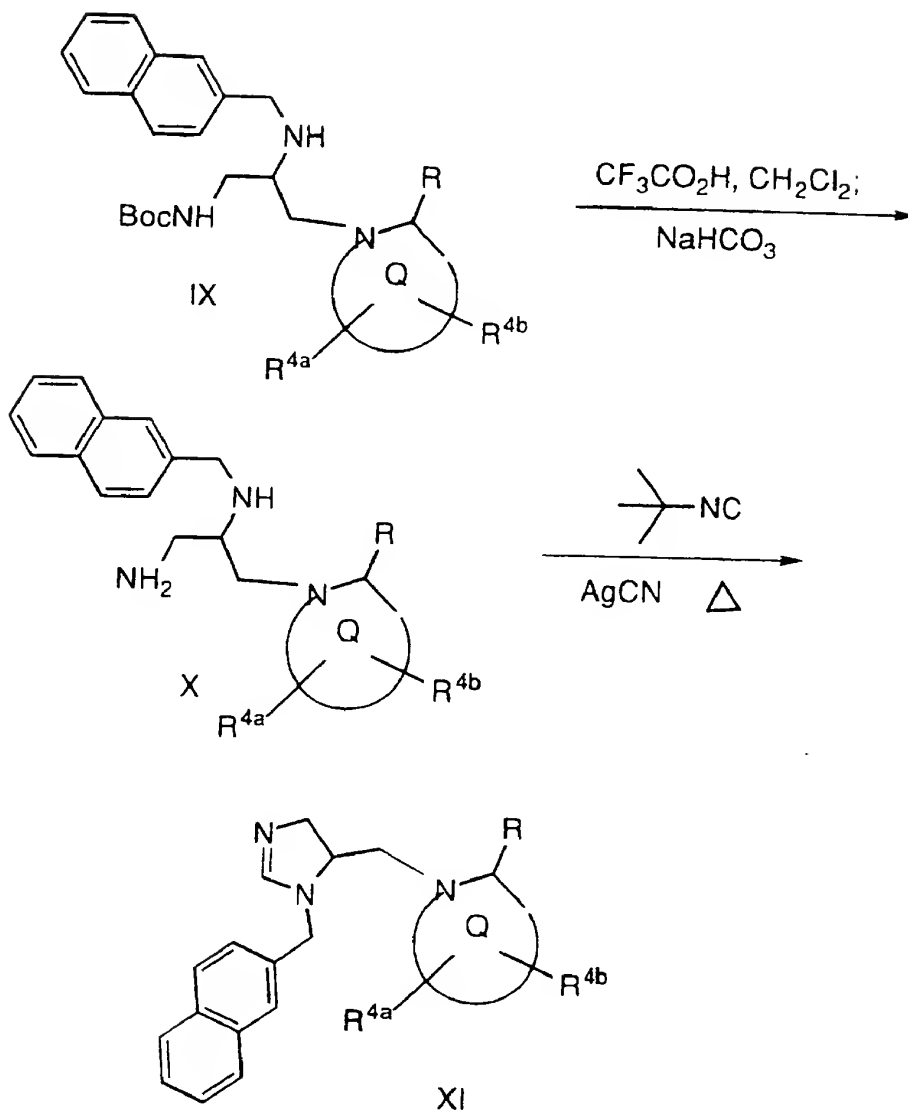
REACTION SCHEME J



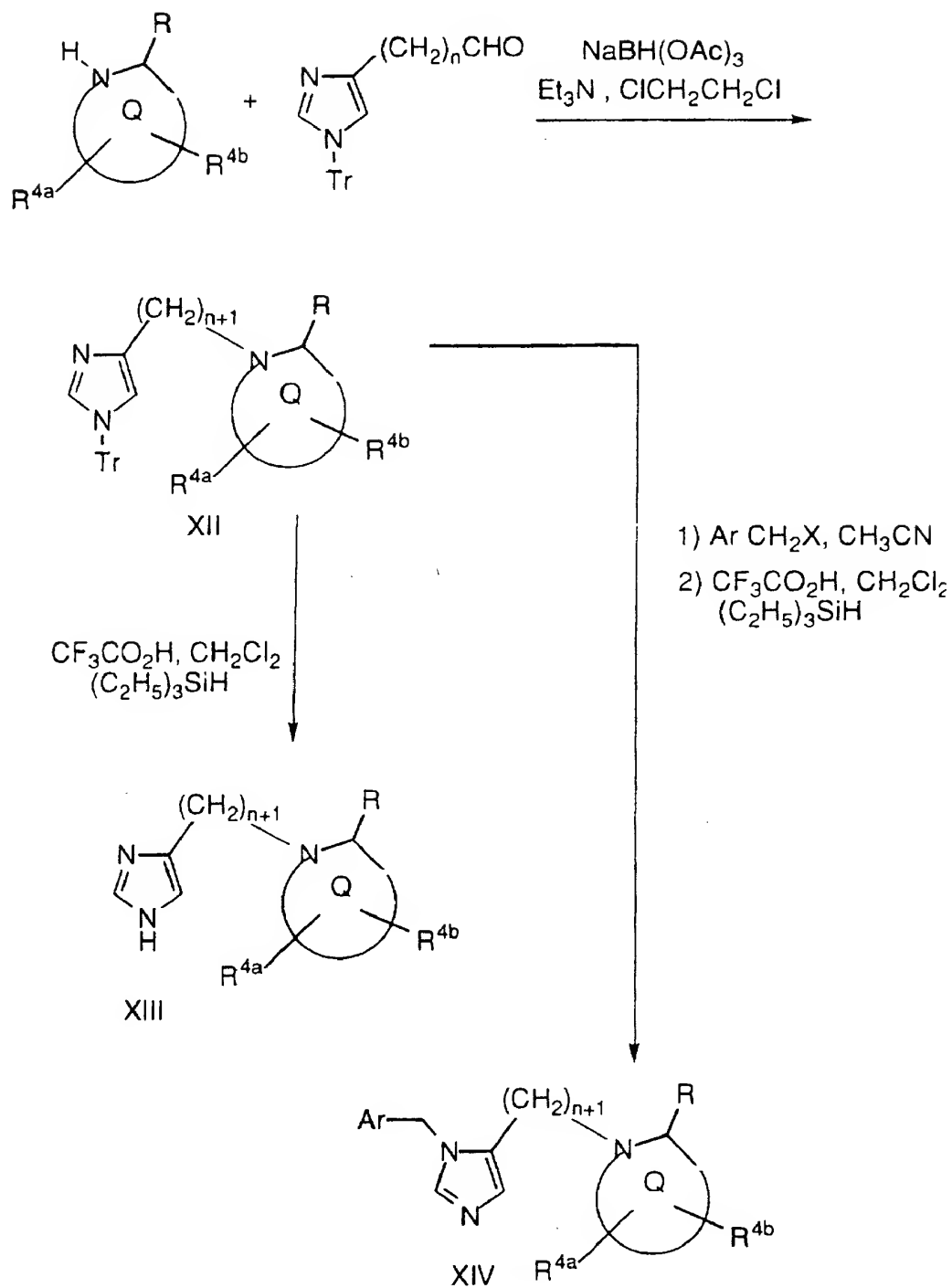
wherein



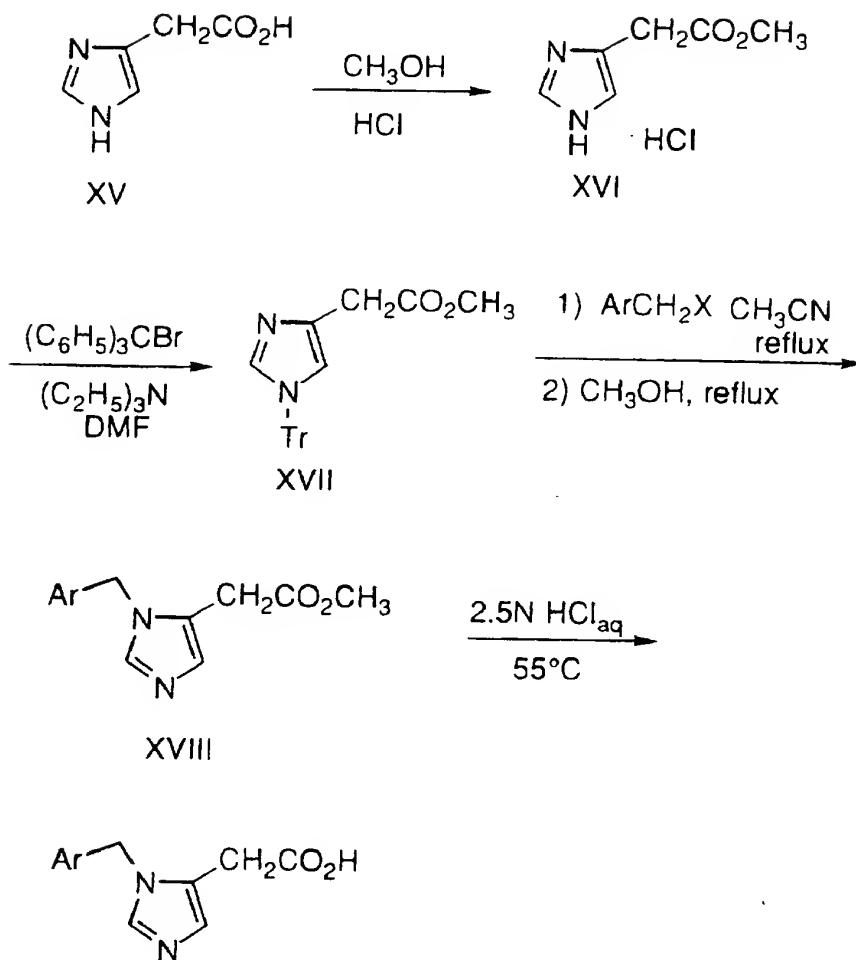
-176-

REACTION SCHEME J (continued)

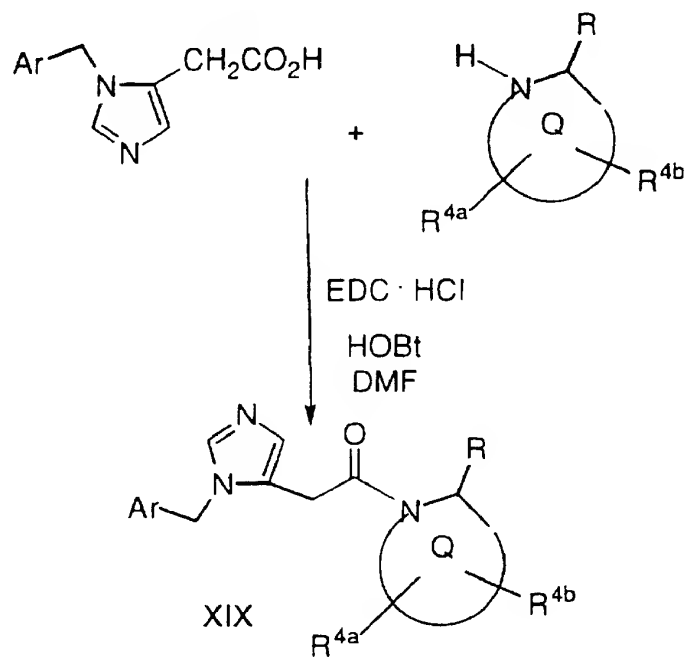
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REACTION SCHEME K

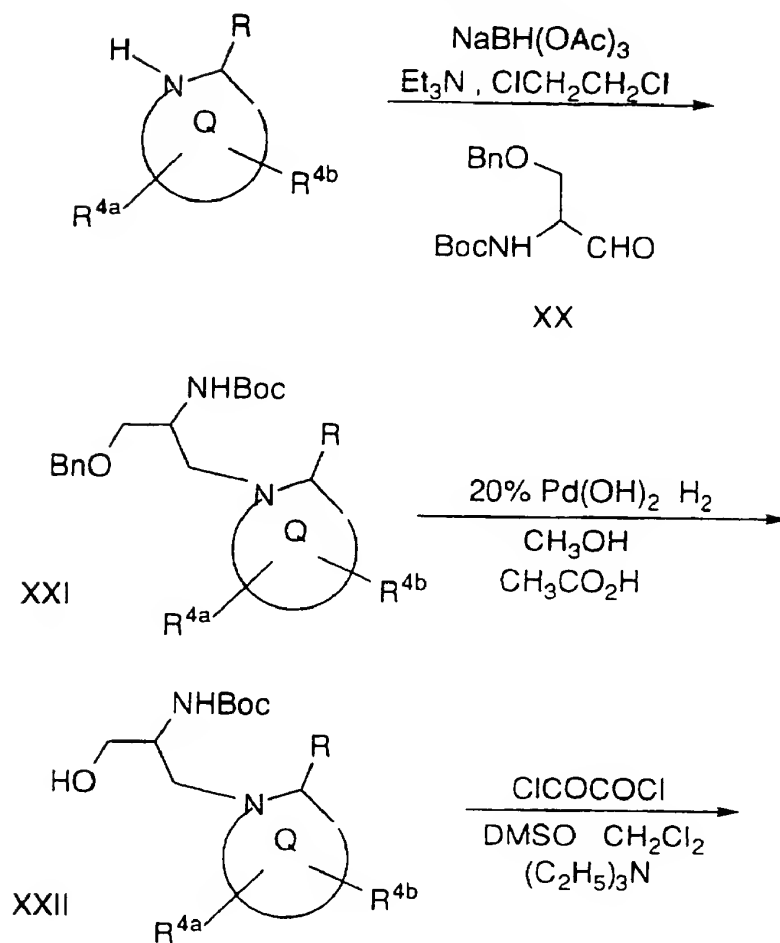
-178-

REACTION SCHEME L

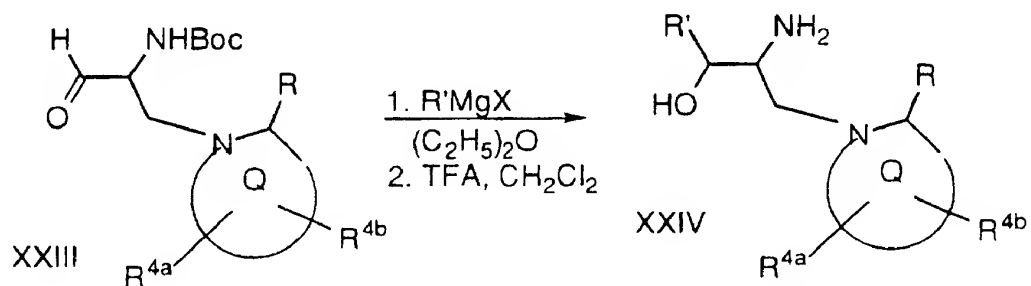
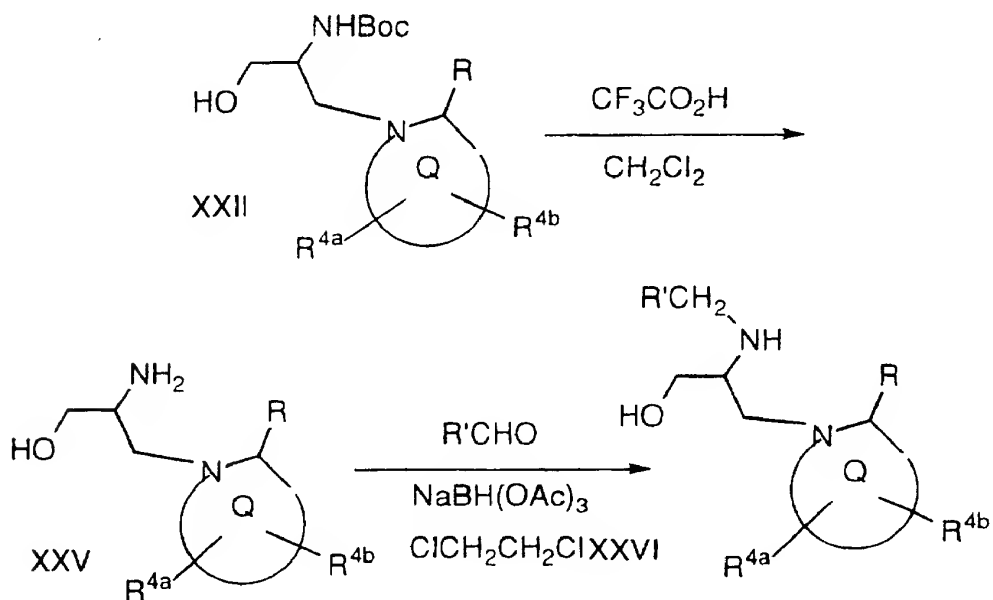
-179-

REACTION SCHEME M

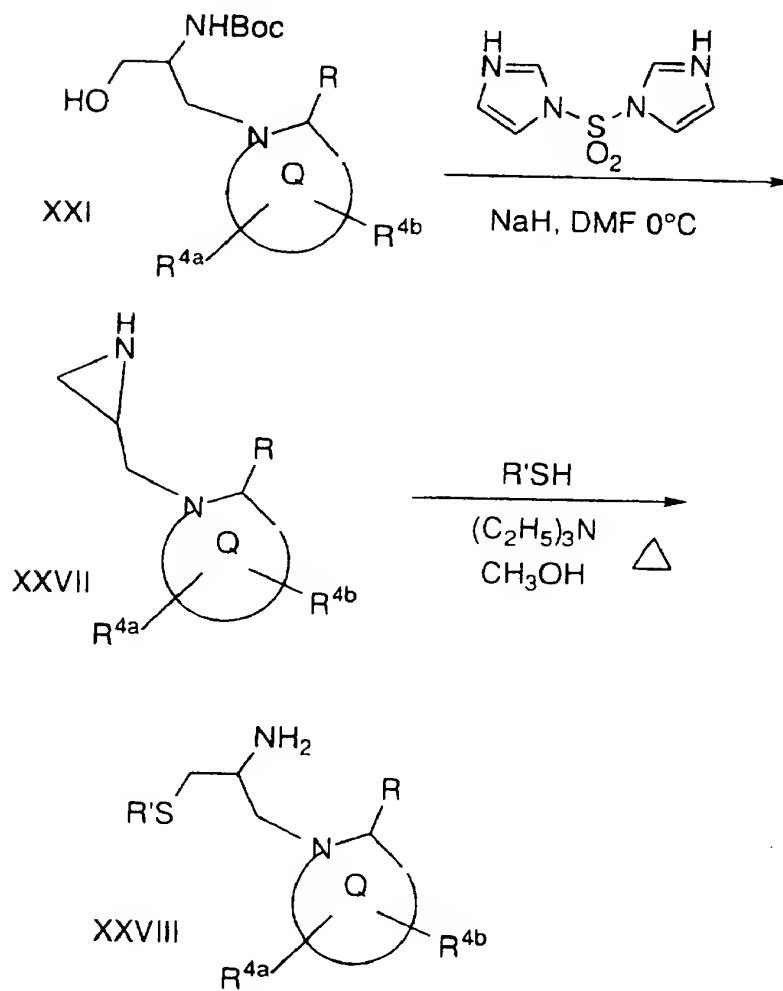
REACTION SCHEME N



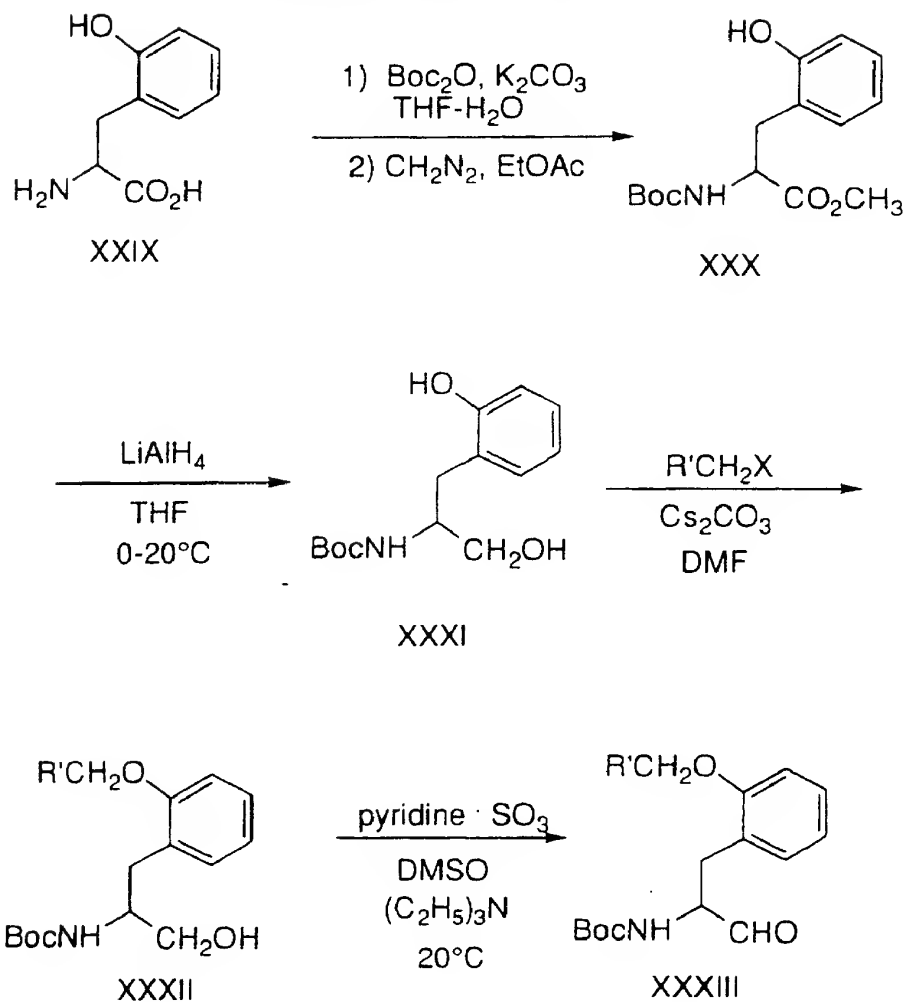
-181-

REACTION SCHEME N (continued)REACTION SCHEME P

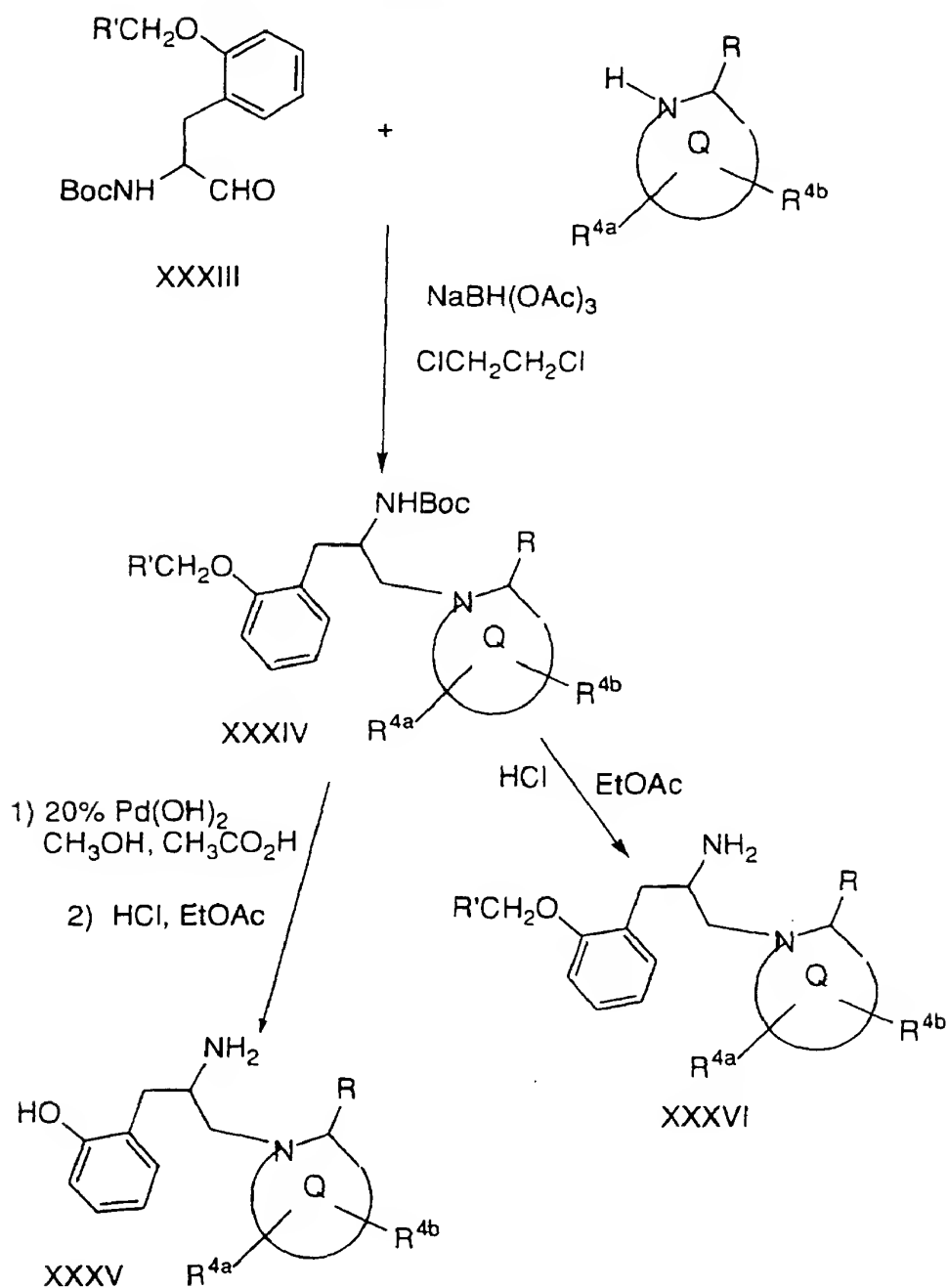
-182-

REACTION SCHEME Q

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REACTION SCHEME R

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REACTION SCHEME B (continued)

The intermediates whose synthesis are illustrated in Reaction Schemes A and C can be reductively alkylated with a variety

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- of aldehydes, such as **I**, as shown in Reaction Scheme F. The aldehydes can be prepared by standard procedures, such as that described by O. P. Goel, U. Krolls, M. Stier and S. Kesten in Organic Syntheses, **1988**, 67, 69-75, from the appropriate amino acid (Reaction Scheme F). The
- 5 reductive alkylation can be accomplished at pH 5-7 with a variety of reducing agents, such as sodium triacetoxyborohydride or sodium cyanoborohydride in a solvent such as dichloroethane, methanol or dimethylformamide. The product **II** can be deprotected to give the final compounds **III** with trifluoroacetic acid in methylene chloride.
- 10 The final product **III** is isolated in the salt form, for example, as a trifluoroacetate, hydrochloride or acetate salt, among others. The product diamine **III** can further be selectively protected to obtain **IV**, which can subsequently be reductively alkylated with a second aldehyde to obtain **V**. Removal of the protecting group, and conversion to
- 15 cyclized products such as the dihydroimidazole **VII** can be accomplished by literature procedures.

- Alternatively, the protected dipeptidyl analog intermediate can be reductively alkylated with other aldehydes such as 1-trityl-4-carboxaldehyde or 1-trityl-4-imidazolylacetaldehyde, to give products
- 20 such as **VIII** (Alternative Reaction Scheme G). The trityl protecting group can be removed from **VIII** to give **IX**, or alternatively, **VIII** can first be treated with an alkyl halide then subsequently deprotected to give the alkylated imidazole **X**. Alternatively, the dipeptidyl analog intermediate can be acylated or sulfonylated by standard techniques.

- 25 The imidazole acetic acid **XI** can be converted to the acetate **XIII** by standard procedures, and **XIII** can be first reacted with an alkyl halide, then treated with refluxing methanol to provide the regiospecifically alkylated imidazole acetic acid ester **XIV** (Alternative Reaction Scheme H). Hydrolysis and reaction with the protected
- 30 dipeptidyl analog intermediate in the presence of condensing reagents such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) leads to acylated products such as **XV**.

If the protected dipeptidyl analog intermediate is reductively alkylated with an aldehyde which also has a protected

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hydroxyl group, such as **XVI** in Reaction Scheme I, the protecting groups can be subsequently removed to unmask the hydroxyl group (Reaction Schemes I, J). The alcohol can be oxidized under standard conditions to *e.g.* an aldehyde, which can then be reacted with a variety
5 of organometallic reagents such as Grignard reagents, to obtain secondary alcohols such as **XX**. In addition, the fully deprotected amino alcohol **XXI** can be reductively alkylated (under conditions described previously) with a variety of aldehydes to obtain secondary amines, such as **XXII** (Reaction Scheme K), or tertiary amines.

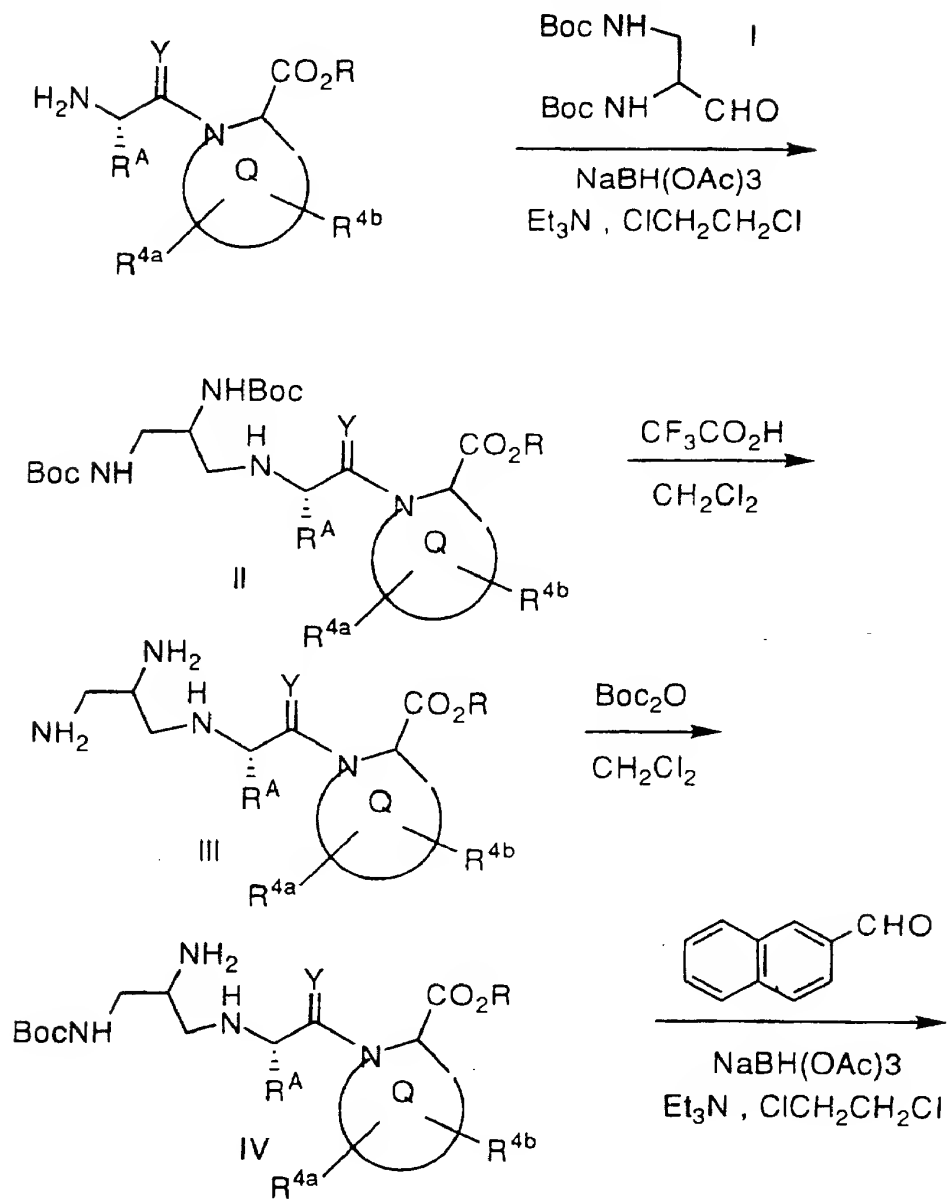
10 The Boc protected amino alcohol **XVIII** can also be utilized to synthesize 2-aziridinylmethylpiperazines such as **XXIII** (Reaction Scheme L). Treating **XVIII** with 1,1'-sulfonyldiimidazole and sodium hydride in a solvent such as dimethylformamide led to the formation of aziridine **XXIII**. The aziridine reacted in the
15 presence of a nucleophile, such as a thiol, in the presence of base to yield the ring-opened product **XXIV**.

In addition, the protected dipeptidyl analog intermediate can be reacted with aldehydes derived from amino acids such as O-alkylated tyrosines, according to standard procedures, to obtain
20 compounds such as **XXX**, as shown in Reaction Scheme M. When R' is an aryl group, **XXX** can first be hydrogenated to unmask the phenol, and the amine group deprotected with acid to produce **XXXI**. Alternatively, the amine protecting group in **XXX** can be removed, and O-alkylated phenolic amines such as **XXXII** produced.

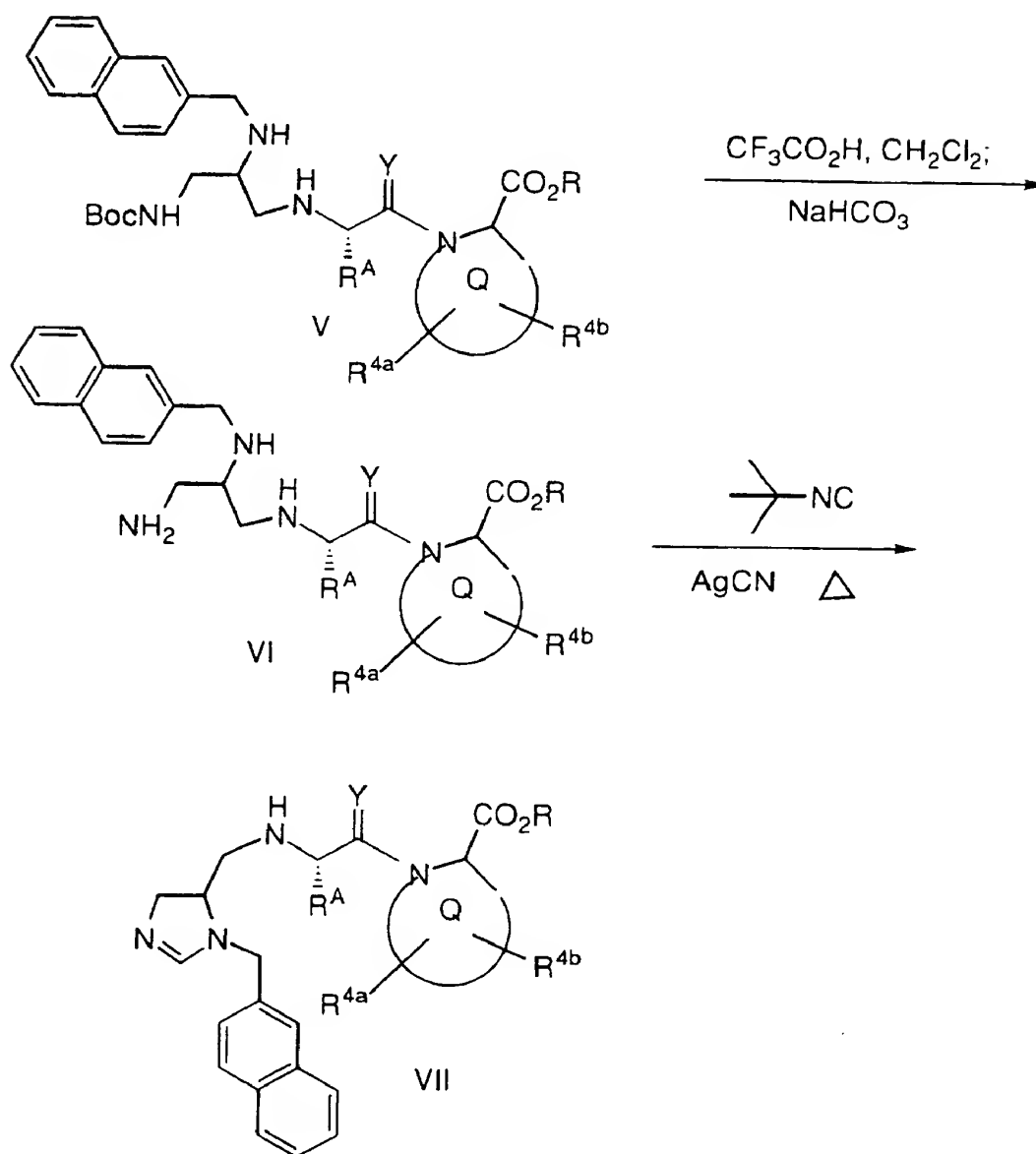
25 Similar procedures as are illustrated in Reaction Schemes F-M may be employed using other peptidyl analog intermediates such as those whose synthesis is illustrated in Reaction Schemes B - E.

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ALTERNATE REACTION SCHEME F FOR
COMPOUNDS (II-h) THROUGH (II-o)

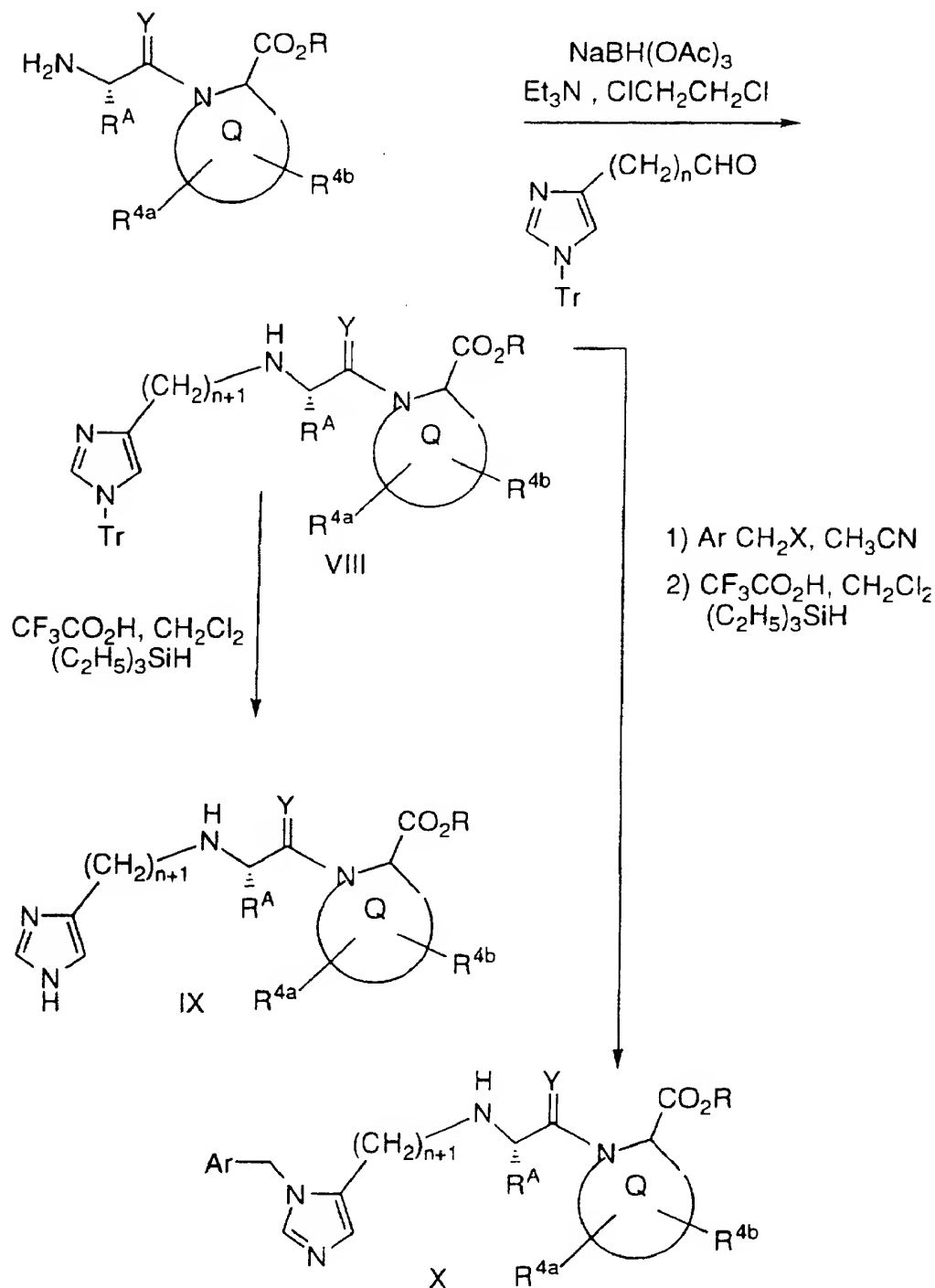


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ALTERNATE REACTION SCHEME F (continued)

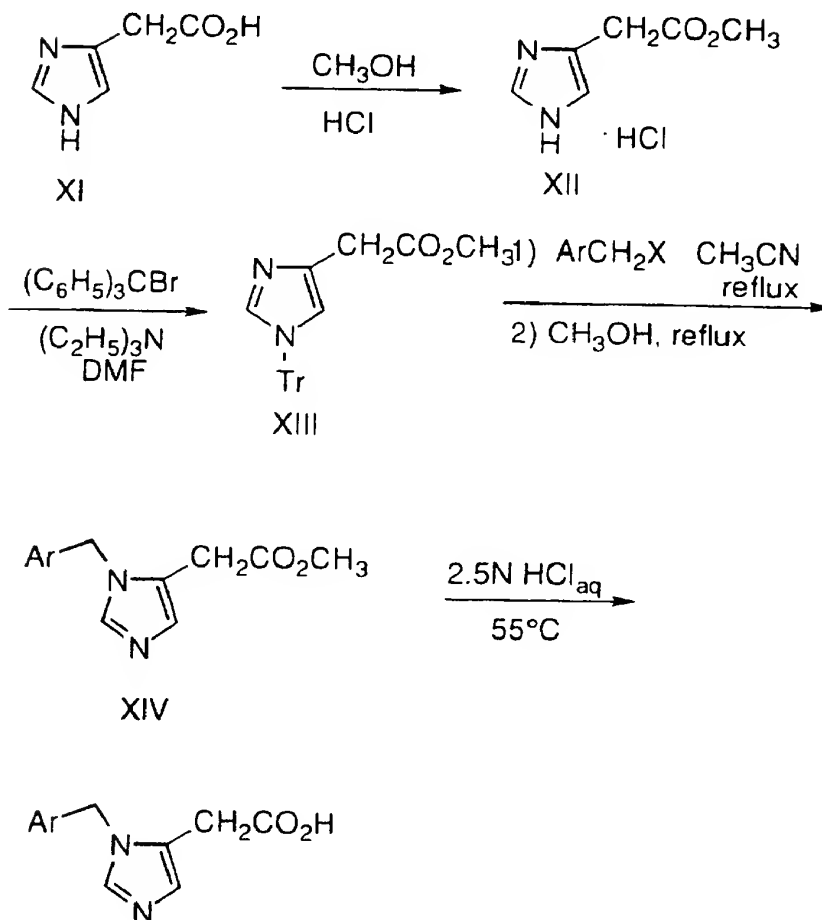
-189-

ALTERNATE REACTION SCHEME G FOR
COMPOUNDS (II-h) THROUGH (II-o)



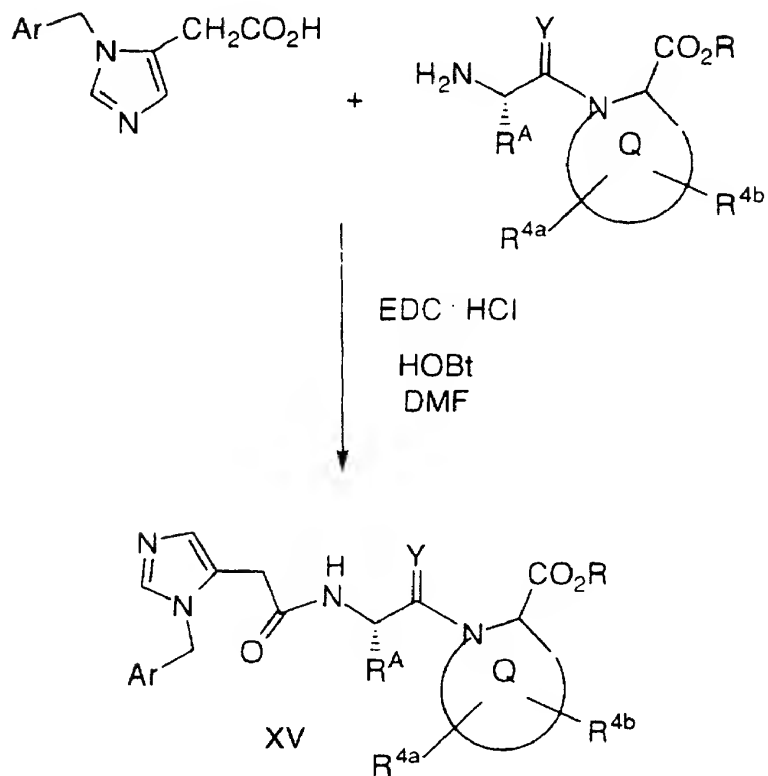
-190-

ALTERNATE REACTION SCHEME H FOR
COMPOUNDS (II-h) THROUGH (II-o)



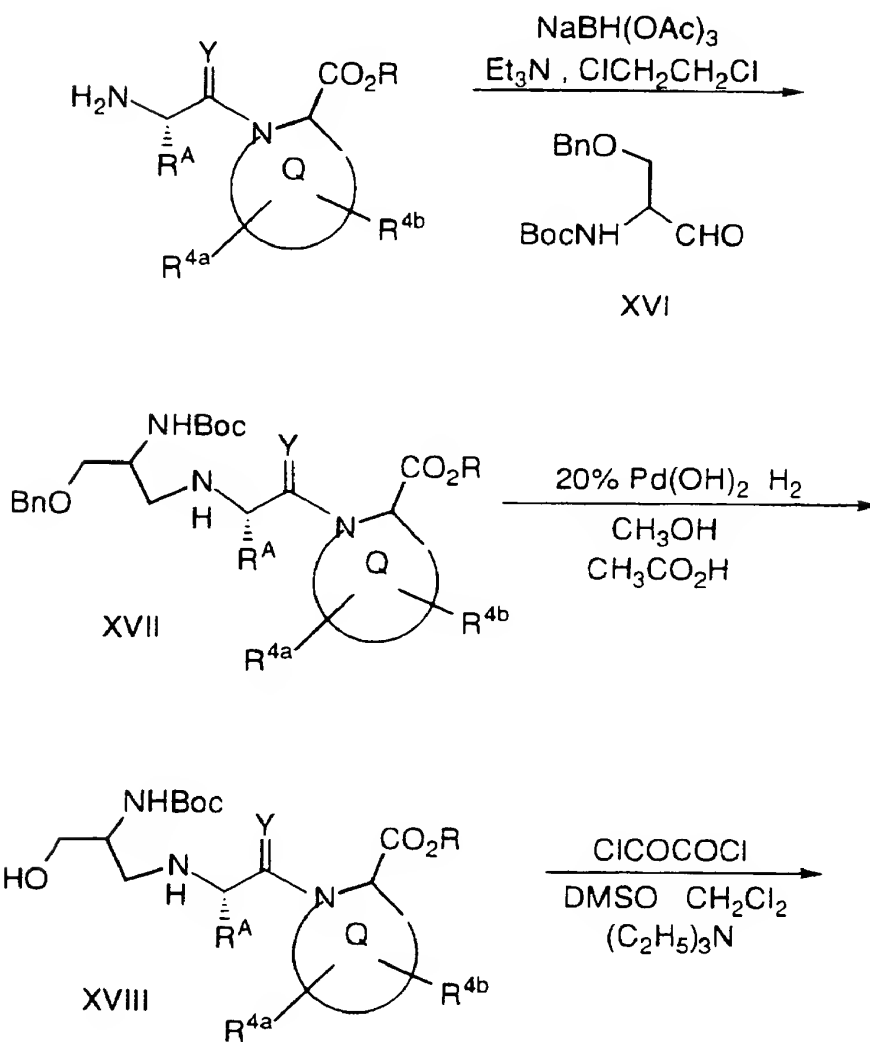
-191-

ALTERNATE REACTION SCHEME I FOR
COMPOUNDS (II-h) THROUGH (II-o)

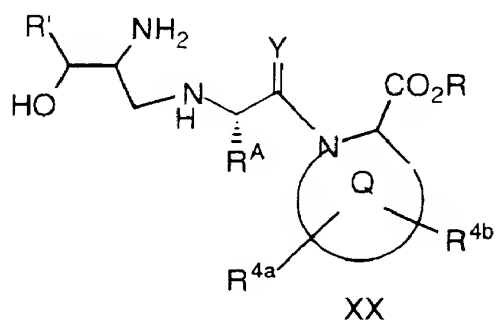
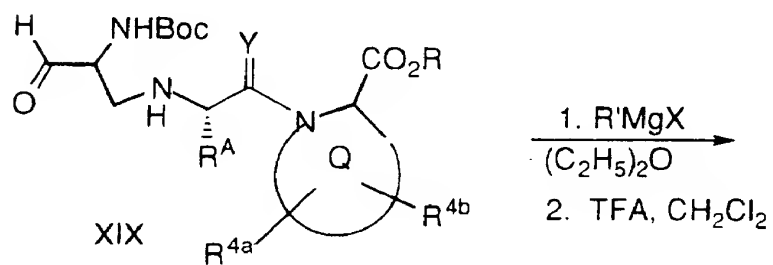


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ALTERNATE REACTION SCHEME J FOR
COMPOUNDS (II-h) THROUGH (II-o)

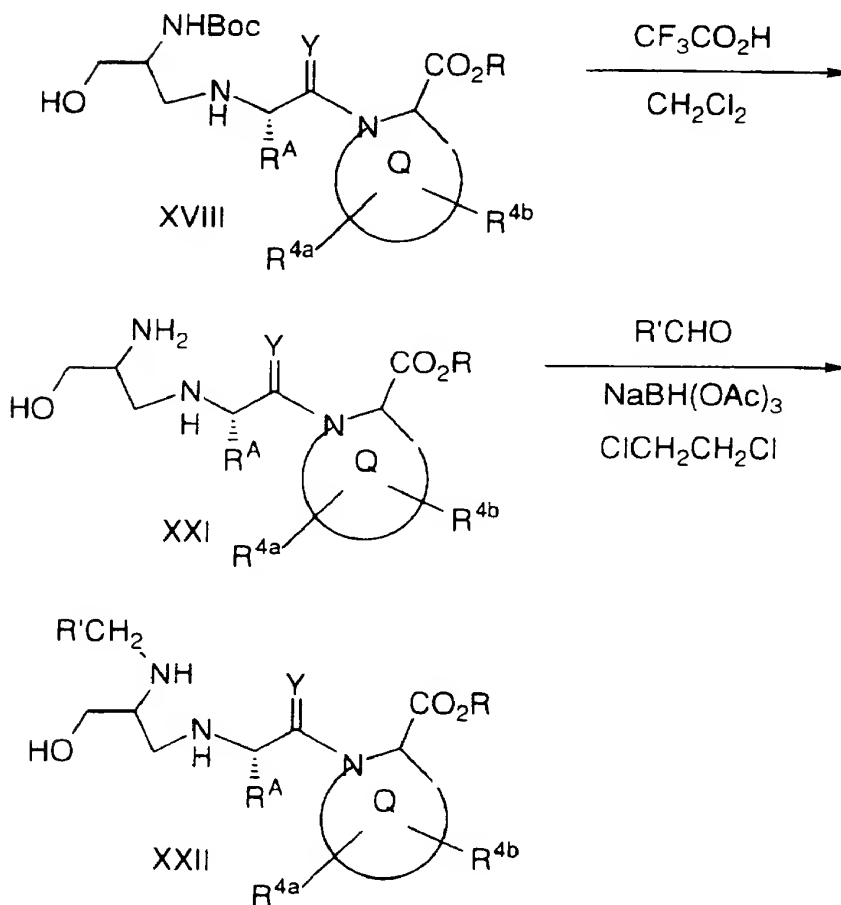


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ALTERNATIVE REACTION SCHEME J (continued)

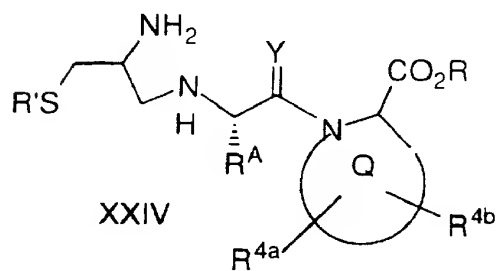
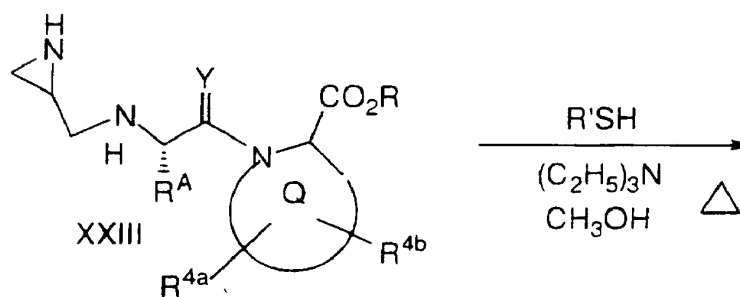
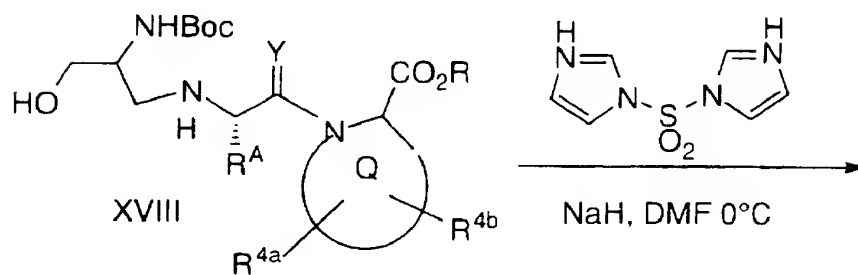
-194-

ALTERNATE REACTION SCHEME K FOR
COMPOUNDS (II-h) THROUGH (II-o)



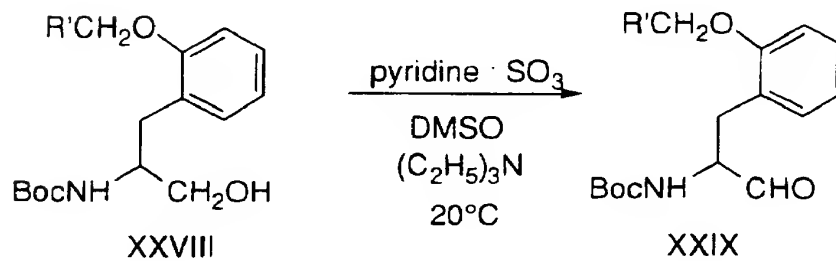
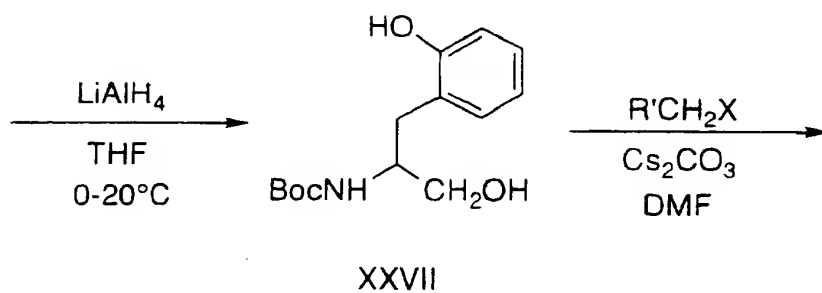
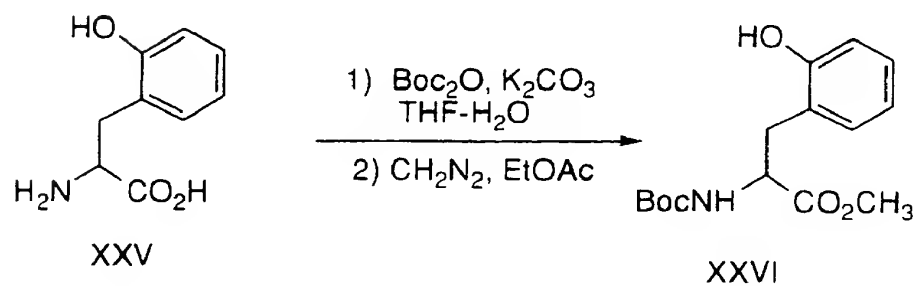
-195-

ALTERNATE REACTION SCHEME I FOR
COMPOUNDS (II-h) THROUGH (II-o)

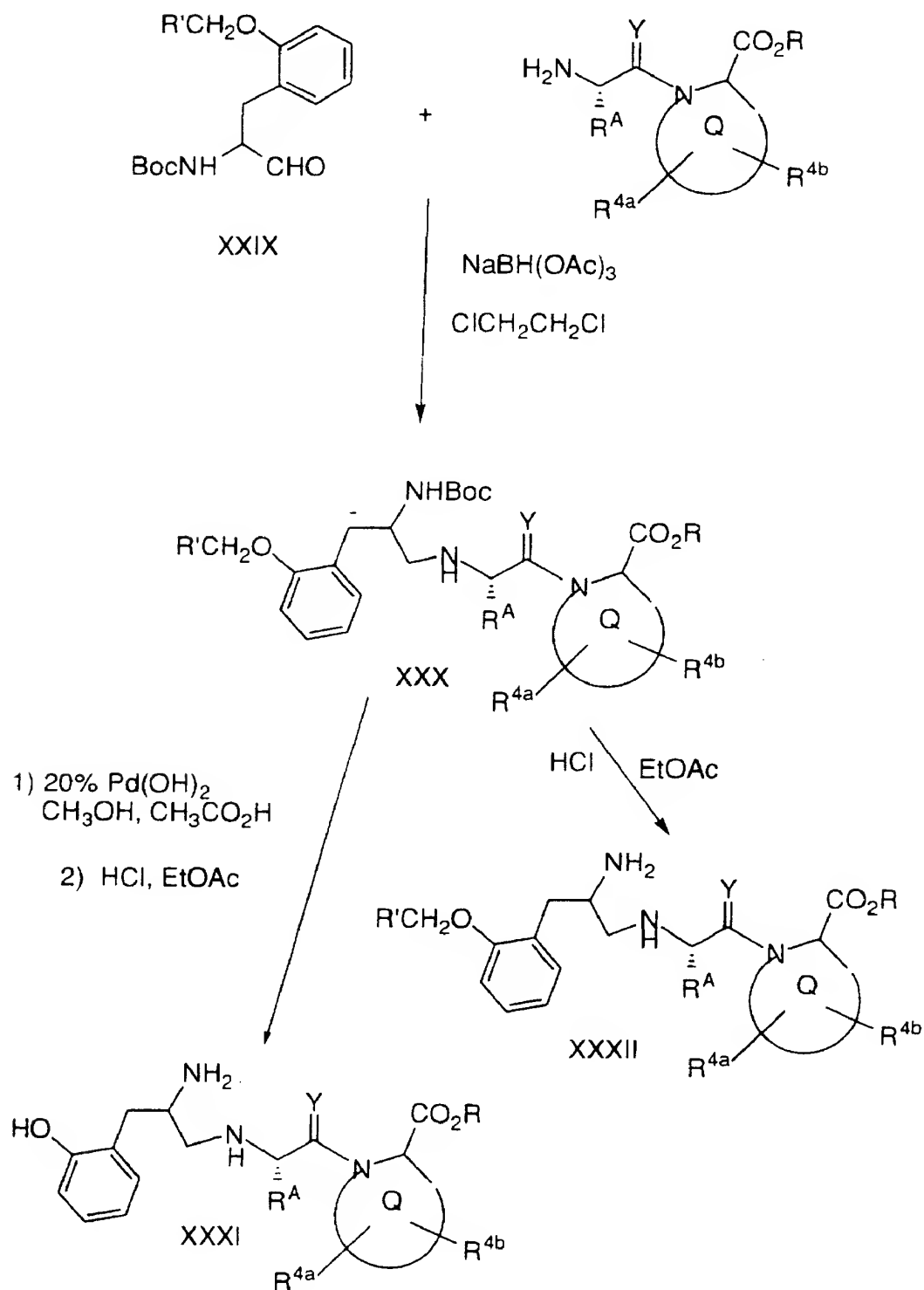


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ALTERNATE REACTION SCHEME M FOR
COMPOUNDS (II-h) THROUGH (II-o)



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ALTERNATE REACTION SCHEME M (CONT.)

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Certain compounds used in the invention are described below.

EXAMPLE 1

5

(S)-1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)-imidazolylmethyl]-5-[2-(methanesulfonyl)ethyl]-2-piperazinone dihydrochloride

Step A: 1-triphenylmethyl-4-(hydroxymethyl)-imidazole

10

To a solution of 4-(hydroxymethyl)imidazole hydrochloride (35.0 g, 260 mmol) in 250 mL of dry DMF at room temperature was added triethylamine (90.6 mL, 650 mmol). A white solid precipitated from the solution. Chlorotriphenylmethane (76.1 g, 273 mmol) in 500 mL of DMF was added dropwise. The reaction mixture was stirred for 20 hours, poured over ice, filtered, and washed with ice water. The resulting product was slurried with cold dioxane, filtered, and dried *in vacuo* to provide the titled product as a white solid which was sufficiently pure for use in the next step.

20

Step B: 1-triphenylmethyl-4-(acetoxymethyl)-imidazole

Alcohol from Step A (260 mmol, prepared above) was suspended in 500 mL of pyridine. Acetic anhydride (74 mL, 780 mmol) was added dropwise, and the reaction was stirred for 48 hours during which it became homogeneous. The solution was poured into 2 L of EtOAc, washed with water (3 x 1 L), 5% aq. HCl soln. (2 x 1 L), sat. aq. NaHCO₃, and brine, then dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the crude product. The acetate was isolated as a white powder which was sufficiently pure for use in the next reaction.

30

Step C: 1-(4-cyanobenzyl)-5-(acetoxymethyl)-imidazole hydrobromide

A solution of the product from Step B (85.8 g, 225 mmol) and α -bromo-*p*-tolunitrile (50.1 g, 232 mmol) in 500 mL of EtOAc was stirred at 60°C for 20 hours, during which a pale yellow precipitate

35

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formed. The reaction was cooled to room temperature and filtered to provide the solid imidazolium bromide salt. The filtrate was concentrated *in vacuo* to a volume 200 mL, reheated at 60°C for two hours, cooled to room temperature, and filtered again. The filtrate was
5 concentrated *in vacuo* to a volume 100 mL, reheated at 60°C for another two hours, cooled to room temperature, and concentrated *in vacuo* to provide a pale yellow solid. All of the solid material was combined, dissolved in 500 mL of methanol, and warmed to 60°C. After two
10 hours, the solution was reconcentrated *in vacuo* to provide a white solid which was triturated with hexane to remove soluble materials. Removal of residual solvents *in vacuo* provided the titled product hydrobromide as a white solid which was used in the next step without further purification.

15 Step D: 1-(4-cyanobenzyl)-5-(hydroxymethyl)-imidazole

To a solution of the acetate from Step C (50.4 g, 150 mmol) in 1.5 L of 3:1 THF/water at 0°C was added lithium hydroxide monohydrate (18.9 g, 450 mmol). After one hour, the reaction was concentrated *in vacuo*, diluted with EtOAc (3 L), and washed with
20 water, sat. aq. NaHCO₃ and brine. The solution was then dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the crude product as a pale yellow fluffy solid which was sufficiently pure for use in the next step without further purification.

25 Step E: 1-(4-cyanobenzyl)-5-imidazolecarboxaldehyde

To a solution of the alcohol from Step D (21.5 g, 101 mmol) in 500 mL of DMSO at room temperature was added triethylamine (56 mL, 402 mmol), then SO₃-pyridine complex (40.5 g, 254 mmol). After 45 minutes, the reaction was poured into 2.5 L of EtOAc.
30 washed with water (4 x 1 L) and brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the aldehyde as a white powder which was sufficiently pure for use in the next step without further purification.

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Step F: (S)-2-(tert-butoxycarbonylamino)-N-methoxy-N-methyl-4-(methylthio)butanamide

L-N-Boc-methionine (30.0 g, 0.120 mol), *N,O*-dimethylhydroxylamine hydrochloride (14.1 g, 0.144 mol), EDC hydrochloride (27.7 g, 0.144 mol) and HOBT (19.5 g, 0.144 mol) were stirred in dry DMF (300 mL) at 20°C under nitrogen. More *N,O*-dimethylhydroxylamine hydrochloride (2.3 g, 23 mmol) was added to obtain pH 7-8. The reaction was stirred overnight, the DMF distilled to half the original volume under high vacuum, and the residue partitioned between ethyl acetate and sat. NaHCO₃ soln. The organic phase was washed with saturated sodium bicarbonate, water, 10% citric acid, and brine, and dried with sodium sulfate. The solvent was removed *in vacuo* to give the title compound.

15 Step G: (S)-2-(tert-butoxycarbonylamino)-4-(methylthio)butanal

A suspension of lithium aluminum hydride (5.02 g, 0.132 mol) in ether (500 mL) was stirred at room temperature for one hour. The solution was cooled to -50°C under nitrogen, and a solution of the product from Step F (39.8 g, ca. 0.120 mol) in ether (200 mL) was added over 30 min, maintaining the temperature below -40°C. When the addition was complete, the reaction was warmed to 5°C, then recooled to -45°C. Analysis by tlc revealed incomplete reaction. The solution was rewarmed to 5°C, stirred for 30 minutes, then cooled to -50°C. A solution of potassium hydrogen sulfate (72 g, 0.529 mol) in 200 mL water was slowly added, maintaining the temperature below -20°C. The mixture was warmed to 5°C, filtered through Celite, and concentrated *in vacuo* to provide the title aldehyde.

30 Step H: (S)-2-(tert-butoxycarbonylamino)-N-(3-chlorophenyl)-4-(methylthio)butanamine

To a solution of 3-chloroaniline (10.3 mL, 97.4 mmol), the product from Step G (23.9 g, 97.4 mmol), and acetic acid (27.8 mL, 487 mmol) in dichloroethane (250 mL) under nitrogen was added sodium triacetoxyborohydride (41.3 g, 195 mmol). The reaction was

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stirred overnight, then quenched with saturated sodium bicarbonate solution. The solution was diluted with CHCl_3 , and the organic phase was washed with water, 10% citric acid and brine. The solution was dried over sodium sulfate and concentrated *in vacuo* to provide the crude product (34.8 g) which was chromatographed on silica gel with 20% ethyl acetate in hexane to obtain the title compound.

Step I: (S)-4-(tert-butoxycarbonyl)-1-(3-chlorophenyl)-5-[2-(methylthio)ethyl]piperazin-2-one

10 A solution of the product from Step H (22.0 g, 63.8 mmol) in ethyl acetate (150 mL) was vigorously stirred at 0°C with saturated sodium bicarbonate (150 mL). Chloroacetyl chloride (5.6 mL, 70.2 mmol) was added dropwise, and the reaction stirred at 0°C for 2h. The layers were separated, and the ethyl acetate phase was washed
15 with 10% citric acid and saturated brine, and dried over sodium sulfate. After concentration *in vacuo*, the resulting crude product (27.6 g) was dissolved in DMF (300 mL) and cooled to 0°C under argon. Cesium carbonate (63.9 g, 196 mmol) was added, and the reaction was stirred for two days, allowing it to warm to room temperature. Another
20 portion of cesium carbonate (10 g, 30 mmol) was added, and the reaction was stirred for 16 hours. The DMF was distilled *in vacuo*, and the residue partitioned between ethyl acetate and water. The organic phase was washed with saturated brine, and dried over sodium sulfate. The crude product was chromatographed on silica gel with 20-25%
25 ethyl acetate in hexane to obtain the title compound.

Step J: (S)-4-(tert-butoxycarbonyl)-1-(3-chlorophenyl)-5-[2-(methanesulfonyl)ethyl]piperazin-2-one

30 A solution of the product from Step I (14.2 g, 37 mmol) in methanol (300 mL) was cooled to 0 °C, and a solution of magnesium monoperoxyphthalate (54.9 g, 111 mmol) in 210 mL MeOH was added over 20 minutes. The ice bath was removed, and the solution was allowed to warm to room temperature. After 45 minutes, the reaction was concentrated *in vacuo* to half the original volume, then quenched by

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the addition of 2N Na₂S₂O₃ soln. The solution was poured into EtOAc and sat NaHCO₃ solution, and the organic layer was washed with brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the crude sulfone. This material was chromatographed on silica gel with
5 60-100% ethyl acetate in hexane to obtain the titled compound.

Step K: (S)-1-(3-chlorophenyl)-5-[2-(methanesulfonyl)ethyl]piperazin-2-one

Through a solution of Boc-protected piperazinone from
10 Step J (1.39 g, 3.33 mmol) in 30 mL of EtOAc at 0 °C was bubbled anhydrous HCl gas. The saturated solution was stirred for 35 minutes, then concentrated *in vacuo* to provide the hydrochloride salt as a white powder. This material was suspended in EtOAc and treated with dilute aqueous NaHCO₃ solution. The aqueous phase was extracted with
15 EtOAc, and the combined organic mixture was washed with brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The resulting amine was reconcentrated from toluene to provide the titled material suitable for use in the next step.

20 Step L: (S)-1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)imidazolylmethyl]-5-[2-(methanesulfonyl)-ethyl]-2-piperazinone dihydrochloride

To a solution of the amine from Step K (898 mg, 2.83 mmol) and imidazole carboxaldehyde from Step E (897 mg, 4.25 mmol)
25 in 15 mL of 1,2-dichloroethane was added sodium triacetoxyborohydride (1.21 g, 5.7 mmol). The reaction was stirred for 23 hours, then quenched at 0 °C with sat. NaHCO₃ solution. The solution was poured into CHCl₃, and the aqueous layer was back-extracted with CHCl₃. The combined organics were washed with brine, dried
30 (Na₂SO₄), filtered, and concentrated *in vacuo*. The resulting product was purified by silica gel chromatography (95:5:0.5-90:10:0 EtOAc:MeOH:NH₄Cl), and the resultant product was taken up in EtOAc/methanol and treated with 2.1 equivalents of 1 M HCl/ether

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solution. After concentrated *in vacuo*, the product dihydrochloride was isolated as a white powder.

EXAMPLE 2

5

1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)imidazolyl-methyl]-2-piperazinone dihydrochloride

Step A: N-(3-chlorophenyl)ethylenediamine hydrochloride
10 To a solution of 3-chloroaniline (30.0 mL, 284 mmol) in 500 mL of dichloromethane at 0°C was added dropwise a solution of 4 N HCl in 1,4-dioxane (80 mL, 320 mmol HCl). The solution was warmed to room temperature, then concentrated to dryness *in vacuo* to provide a white powder. A mixture of this powder
15 with 2-oxazolidinone (24.6 g, 282 mmol) was heated under nitrogen atmosphere at 160°C for 10 hours, during which the solids melted, and gas evolution was observed. The reaction was allowed to cool, forming the crude diamine hydrochloride salt as a pale brown solid.

20 Step B: N-(tert-butoxycarbonyl)-N'-(3-chlorophenyl)ethylenediamine

The amine hydrochloride from Step A (*ca.* 282 mmol, crude material prepared above) was taken up in 500 mL of THF and 500 mL of sat. aq. NaHCO₃ soln., cooled to 0°C, and di-*tert*-
25 butylpyrocarbonate (61.6 g, 282 mmol) was added. After 30 h, the reaction was poured into EtOAc, washed with water and brine, dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the titled carbamate as a brown oil which was used in the next step without further purification.

30

Step C: N-[2-(tert-butoxycarbamoyl)ethyl]-N-(3-chlorophenyl)-2-chloroacetamide

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A solution of the product from Step B (77 g, *ca.* 282 mmol) and triethylamine (67 mL, 480 mmol) in 500 mL of CH₂Cl₂ was cooled to 0°C. Chloroacetyl chloride (25.5 mL, 320 mmol) was added dropwise, and the reaction was maintained at 0°C with stirring. After 3 h,
5 another portion of chloroacetyl chloride (3.0 mL) was added dropwise. After 30 min, the reaction was poured into EtOAc (2 L) and washed with water, sat. aq. NH₄Cl soln, sat. aq. NaHCO₃ soln., and brine. The solution was dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the chloroacetamide as a brown oil which was used in the
10 next step without further purification.

Step D: 4-(*tert*-butoxycarbonyl)-1-(3-chlorophenyl)-2-piperazinone

To a solution of the chloroacetamide from Step C (*ca.* 282
15 mmol) in 700 mL of dry DMF was added K₂CO₃ (88 g, 0.64 mol). The solution was heated in an oil bath at 70-75°C for 20 hours, cooled to room temperature, and concentrated *in vacuo* to remove *ca.* 500 mL of DMF. The remaining material was poured into 33% EtOAc/hexane, washed with water and brine, dried (Na₂SO₄), filtered, and concentrated
20 *in vacuo* to provide the product as a brown oil. This material was purified by silica gel chromatography (25-50% EtOAc/hexane) to yield pure product, along with a sample of product (*ca.* 65% pure by HPLC) containing a less polar impurity.

25 Step E: 1-(3-chlorophenyl)-2-piperazinone

Through a solution of Boc-protected piperazinone from Step D (17.19 g, 55.4 mmol) in 500 mL of EtOAc at -78°C was bubbled anhydrous HCl gas. The saturated solution was warmed to 0°C, and stirred for 12 hours. Nitrogen gas was bubbled through the reaction to
30 remove excess HCl, and the mixture was warmed to room temperature. The solution was concentrated *in vacuo* to provide the hydrochloride as a white powder. This material was taken up in 300 mL of CH₂Cl₂ and treated with dilute aqueous NaHCO₃ solution. The aqueous phase was extracted with CH₂Cl₂ (8 x 300 mL) until tlc analysis indicated

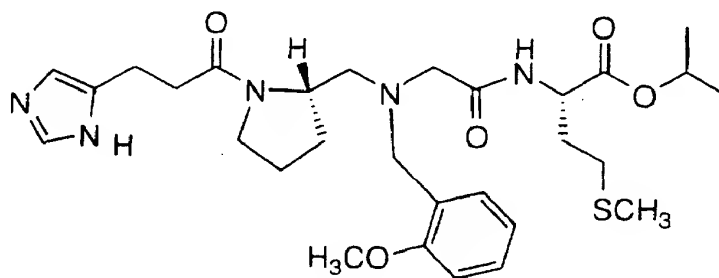
-205-

complete extraction. The combined organic mixture was dried (Na₂SO₄), filtered, and concentrated *in vacuo* to provide the titled free amine as a pale brown oil.

5 Step F: 1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)imidazolylmethyl]-
2-piperazinone dihydrochloride

To a solution of the amine from Step E (55.4 mmol, prepared above) in 200 mL of 1,2-dichloroethane at 0°C was added 4Å powdered molecular sieves (10 g), followed by sodium triacetoxyborohydride (17.7 g, 83.3 mmol). The imidazole carboxaldehyde from Step
10 E of Example 1 (11.9 g, 56.4 mmol) was added, and the reaction was stirred at 0°C. After 26 hours, the reaction was poured into EtOAc, washed with dilute aq. NaHCO₃, and the aqueous layer was back-extracted with EtOAc. The combined organics were washed with brine,
15 dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The resulting product was taken up in 500 mL of 5:1 benzene:CH₂Cl₂, and propylamine (20 mL) was added. The mixture was stirred for 12 hours, then concentrated *in vacuo* to afford a pale yellow foam. This material was purified by silica gel chromatography (2-7% MeOH/CH₂Cl₂), and the
20 resultant white foam was taken up in CH₂Cl₂ and treated with 2.1 equivalents of 1 M HCl/ether solution. After concentrated *in vacuo*, the product dihydrochloride was isolated as a white powder.

EXAMPLE 3



25 N-[1-(1H-imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester

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Step A: 2-Methoxybenzylglycine methyl ester

2-Methoxybenzyl alcohol (53.5 g, 0.39 mol) was dissolved in CH₂Cl₂ (200 mL), treated with diisopropylethylamine (81 mL, 0.74 mol), cooled to 0°C. with stirring in an ice-CH₃OH bath under Ar, and treated dropwise with methanesulfonyl chloride (33 mL, 0.43 mol). After 0.5 hr, the reaction mixture was left to warm to ambient temperature and stirred for 4 hr. This solution and diisopropylethylamine (202.5 mL, 1.16 mol) were added alternately portionwise with to a slurry of glycine methyl ester hydrochloride (146.5 g, 1.17 mol) in DMF (250 mL) with stirring at 0°C. The reaction mixture was left to stir and warm to room temperature overnight. The DMF was removed under reduced pressure, and the residue was partitioned between EtOAc (1 L) and satd NaHCO₃ solution (1 L). The aqueous layer was washed with EtOAc (2 x 600 mL), the organics combined, washed with brine and dried (MgSO₄). Filtration and concentration to dryness gave the title compound after chromatography (SiO₂, 1-5% CH₃OH/CH₂Cl₂).

Step B: N-[(2S)-(t-Butoxycarbonylpyrrolidinyl-methyl)-N-(2-methoxybenzyl)glycine methyl ester

2-Methoxybenzylglycine methyl ester (27.4 g, 0.131 mol) was dissolved in 1,2-dichloroethane (500 ml), 3 Å molecular sieves (20 g) were added, and the pH of the reaction mixture adjusted to pH 5 with acetic acid (7.5 mL, 0.131 mol). N-(t-Butoxycarbonyl)-L-proline (26.1 g, 0.131 mol) (J. Org. Chem. (1994) **59**, [21], 6287-95) was added followed by sodium triacetoxyborohydride (33.2 g, 0.157 mol). The mixture was stirred at ambient temperature for 18 h, filtered through celite and concentrated. The residue was partitioned between EtOAc and sat. NaHCO₃ (500 ml/100 ml). The aqueous layer was washed with EtOAc (3x100 ml). The organic layers were combined, dried with Na₂SO₄, filtered, and concentrated to give the title compound.

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Step C: N-[(2S)-(t-Butoxycarbonylpyrrolidinyl-methyl)-N-(2-methoxybenzyl)glycine

 N-[(2S)-(t-Butoxycarbonylpyrrolidinylmethyl)-N-(2-methoxybenzyl)glycine methyl ester (7.0 g, 0.018 mol) was dissolved in
5 CH₃OH (150 ml) and 1N NaOH (71 ml, 0.071 mol) was added with
cooling in an ice-water bath. The mixture was stirred at ambient
temperature for 2 hr, neutralized with 1N HCl (71 ml, 0.071 mol),
concentrated to remove the CH₃OH, and the residue extracted with
EtOAc (3x200 mL). The organic layers were combined, dried with
10 MgSO₄, filtered, and concentrated to give the title compound as a
foam.

Step D: Methionine isopropyl ester hydrochloride

 N-(t-Butoxycarbonyl)methionine (25 g, 0.1 mol), isopropyl
15 alcohol (11.8 mL, 0.15 mol), EDC (21.1 g, 0.11 mol), and 4-dimethyl-
aminopyridine (DMAP) (1.22 g, 0.01 mol) were dissolved in CH₂Cl₂
(400 mL) with stirring in an ice-water bath. After 2 h the bath was
removed, and the solution was left to stir o.n. at RT. The reaction
mixture was concentrated to dryness, then partitioned between EtOAc
20 and H₂O, the aqueous layer washed with EtOAc (2 x 50 mL), the
organics combined, washed with NaHCO₃ soln, brine, and dried
(Na₂SO₄). Filtration and concentration to dryness gave a yellow oil
after chromatography (flash silica gel column, hexane: EtOAc, 6:1 to
5:1).

25 N-(t-Butoxycarbonyl)methionine isopropyl ester (20.5 g,
0.07 mol) was dissolved in EtOAc (200 mL) with stirring and cooling to
-20°C in a dry ice- acetone bath. HCl gas was bubbled into the solution
for 10 min, the flask was stoppered and stirred for 1 h. Tlc (EtOAc:
hexane, 1:3) indicates loss of starting material. Argon was bubbled
30 through the soln for 5 min, then it was concentrated to dryness to give
the title compound as a white solid.

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Step E: N-[(2S)-(t-Butoxycarbonylpyrrolidinyl-methyl)-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester]
N-[(2S)-(t-Butoxycarbonylpyrrolidinylmethyl)-N-(2-methoxybenzyl)glycine (from step C) (5.98 g, 0.0158 mol), dissolved
5 in CH₂Cl₂ (100 mL), was treated with HOBt (2.57 g, 0.019 mol), EDC (4.54 g, 0.024 mol), and methionine isopropyl ester hydrochloride (4.33 g, 0.019 mol). The pH was adjusted to 7.5 with Et₃N (8.81 mL, 0.063 mol) and the mixture was stirred at ambient temperature for 18 h. The mixture was diluted with EtOAc (150 mL) and washed sequentially with
10 10% citric acid soln, saturated NaHCO₃ solution, brine, and dried (MgSO₄). Filtration and concentration to dryness gave the title compound as a thick oil. This was used without further purification.

Step F: N-((2S)-Pyrrolidinylmethyl)-N-(2-methoxybenzyl)-glycyl-methionine isopropyl ester bis hydrochloride
15 N-[(2S)-(t-Butoxycarbonylpyrrolidinylmethyl)-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester (0.85 g, 1.54 mmol) was dissolved in EtOAc (25 mL) and cooled to 0°C. HCl was bubbled through the mixture until the soln was saturated, and it was stoppered
20 and stirred for 3 hr. Argon was bubbled through the mixture to remove excess HCl and the mixture was then concentrated to give the title compound.

Step G: N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester
25 N-((2S)-Pyrrolidinylmethyl)-N-(2-methoxybenzyl)glycyl methionine isopropyl ester bis hydrochloride (0.800 g, 1.53 mmol), dissolved in DMF (30 mL), was treated with 1H-imidazol-4-propionic acid (0.43 g, 3.05 mmol) (prepared by catalytic hydrogenation of urocanic acid in 20% acetic acid with Pd on carbon), and BOP reagent
30 (1.35 g, 3.05 mmol). The pH was adjusted to 7.5 with N-methyl-morpholine (0.756 mL, 6.89 mmol), and the mixture was stirred

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at ambient temperature for 18 h. The mixture was concentrated to dryness, diluted with EtOAc (100 mL), washed with 5% Na₂CO₃ solution, brine, and dried (MgSO₄). Filtration and concentration to dryness gave an oil which was purified by chromatography (silica gel, 95:5 CH₂Cl₂/MeOH) to give the title compound as a foam.

¹H NMR (CD₃OD); δ 7.58 (d, 1H, J=1 Hz), 7.25-7.31 (m, 2H), 6.89-7.00 (m, 2H), 6.81 (s, 1H), 5.00-5.06 (m, 1H), 4.49-4.56 (m, 1H), 4.23-4.30 (m, 1H), 3.91 (d, 1H, J=13 Hz), 3.86 (s, 3H), 3.54 (d, 1H, J=13 Hz), 3.30-3.41 (m, 2H), 3.36 (d, 1H, J=17 Hz), 3.15 (d, 1H, J=17 Hz), 2.85-2.92 (m, 2H), 2.56-2.77 (m, 3H), 2.30-2.45 (m, 3H), 2.05-2.17 (m, 1H), 2.04 (s, 3H), 1.69-1.86 (m, 5H), 1.24 (d, 6H, J=6 Hz).

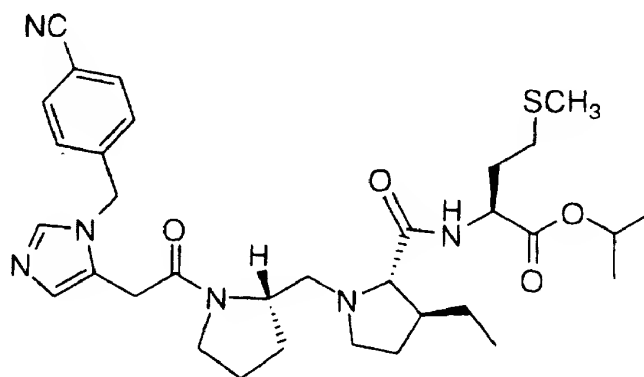
Anal. calculated for C₂₉H₄₃N₅O₅S • 0.6 H₂O:

C, 59.59; H, 7.62; N, 11.98;

Found: C, 59.58; H, 7.43; N, 12.02.

EXAMPLE 4

(N-[1-Cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-
3(S)-ethyl-propyl methionine isopropyl ester



Step A: Diethyl 1-Acetyl-5-hydroxy-3-ethylpyrrolidine-2,2-dicarboxylate

Sodium (4.02 g, 0.175 mol) was dissolved in a stirred solution of diethyl acetamidomalonate (235.4 g, 1.19 mol) in abs EtOH

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(1.4 L) at ambient temperature under argon. The reaction mixture was cooled to 0°C, and trans-2-pentenal (100 g, 1.08 mol) was added dropwise maintaining the reaction temperature at <5°C. After the addition, the reaction was allowed to warm to room temperature, stirred for 4 h, then quenched with acetic acid (28 mL). The solution was concentrated *in vacuo*, and the residue dissolved in EtOAc (1.5 L), washed with 10% NaHCO₃ solution (2 x 300 mL), brine, and dried (MgSO₄). The solution was filtered and concentrated to 700 mL, then heated to reflux and treated with hexane (1 L). On cooling, the title compound precipitated and was collected, mp 106 - 109°C. ¹H NMR (CD₃OD) δ 5.65 (d, 1H, J = 5 Hz), 4.1 - 4.25 (m, 4H), 2.7-2.8 (m, 1H), 2.21 (s, 3H), 2.10 (dd, 1H, J = 6, 13, Hz), 1.86- 1.97 (m, 2H), 1.27 (t, 3H, J = 7 Hz), 1.23 (t, 3H, J = 7 Hz), 1.1- 1.25 (m, 1H), 0.97 (t, 3H, J = 7 Hz).

15 Step B: Diethyl 1-Acetyl-3-ethylpyrrolidine-2,2-dicarboxylate
To a solution of diethyl 1-acetyl-5-hydroxy-3-ethylpyrrolidine-2,2-dicarboxylate (287 g, 0.95 mol) and triethylsilane (228 mL, 1.43 mol) in CH₂Cl₂ (3 L) under argon was added trifluoroacetic acid (735 mL, 9.53 mol) dropwise with stirring while maintaining the internal temperature at 25 °C by means of an ice bath. After stirring for 3 h at 23°C, the solution was concentrated *in vacuo*, the residue diluted with CH₂Cl₂ (1.5 L), then treated with H₂O (1 L) and solid Na₂CO₃ with vigorous stirring until the solution was basic. The organic layer was separated, dried (Na₂SO₄), filtered, then concentrated to give the title compound as a yellow oil which was used without further purification.

Step C: 3-Ethylproline hydrochloride (Cis:Trans Mixture)
Diethyl 1-acetyl-3-ethylpyrrolidine-2,2-dicarboxylate (373 g, 0.95 mol) was suspended in 6N HCl (2 L) and HOAc (500 mL) and heated at reflux for 20 h. The reaction mixture was cooled, washed with EtOAc (1L), then concentrated *in vacuo* to give an oil which crystallized upon trituration with ether to give the title compound.

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^1H NMR (D_2O) δ 4.23 (d, 1H, $J = 8$ Hz), 3.84 (d, 1H, $J = 8$ Hz), 3.15-3.4 (m, 4H), 2.33- 2.44 (m, 1H), 2.19-2.4 (m, 1H), 2.02- 2.15 (m, 2H), 1.53- 1.72 (m, 3H), 1.23- 1.43 (m, 2H), 1.0- 1.15 (m, 1H), 0.75 - 0.83 (m, 6H).

5

Step D: N-[(*tert*-Butyloxy)carbonyl]-*cis:trans*-3-ethylproline methyl ester

3-Ethylproline hydrochloride (Cis:Trans Mixture) (20 g, 0.11 mol) was dissolved in CH_3OH (200 mL), and the solution was saturated with HCl gas, then stirred at 23°C for 24 h. Argon was bubbled through the solution to remove excess HCl. The solution was treated with NaHCO_3 (>84 g) to a pH of 8, then di-*tert*-butyl dicarbonate (25.1 g, 0.115 mol) dissolved in CH_3OH (20 mL) was added slowly. After stirring for 18 h at 23°C , the mixture was filtered, the filtrate concentrated, and the residue triturated with EtOAc, filtered again, and concentrated to give the title compound as an oil.

Step E: N-[(*tert*-Butyloxy)carbonyl]-*trans*-3-ethylproline and N-[(*tert*-Butyloxy)carbonyl]-*cis*-3-ethylproline methyl ester

N-[(*tert*-Butyloxy)carbonyl]-*cis:trans*-3-ethylproline methyl ester (29.1 g, 0.113 mol) was dissolved in CH_3OH (114 mL) with cooling to 0°C , then treated with 1 N NaOH (114 mL). After stirring for 20 h at 23°C , the solution was concentrated to remove the CH_3OH and then extracted with EtOAc (3 x). The organic layers were combined, dried (MgSO_4), filtered, and concentrated to give 12.8 g of N-[(*tert*-Butyloxy)carbonyl]-*cis*-3-ethylproline methyl ester as an oil. The aqueous layer was acidified with solid citric acid and extracted with EtOAc (2 x), the organic layers combined, dried (MgSO_4), filtered, and concentrated to give N-[(*tert*-Butyloxy)carbonyl]-*trans*-3-ethylproline as an oil. ^1H NMR (CD_3OD) δ 3.86 and 3.78 (2 d, 1H, $J = 6$ Hz), 3.33 - 3.58 (m, 2H), 2.01 - 2.22 (m, 2H), 1.5 - 1.74 (m, 2H), 1.33 - 1.5 (m, 1H), 1.45 and 1.42 (2 s, 9H), 0.98 (t, 3H, $J = 8$ Hz).

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Step F: 3(S)-Ethyl-2(S)-proline hydrochloride

N-[(*tert*-Butyloxy)carbonyl]-*trans*-3-ethylproline (15.5 g, 0.064 mol), S- α -methylbenzylamine (9.03 mL, 0.070 mol), HOBT (10.73 g, 0.070 mol), and N-methylmorpholine (8 mL, 0.076 mol) were dissolved in CH₂Cl₂ (150 mL) with stirring in an ice-H₂O bath, treated with EDC (13.4 g, 0.070 mol) stirred at 23°C for 48 h. The reaction mixture was partitioned between EtOAc and 10% citric acid solution, the organic layer washed with satd NaHCO₃ solution, brine and dried (MgSO₄), filtered, and concentrated to give an oil. This oil was dissolved in a minimum amount of ether (10 mL) to crystallize the desired S,S,S diastereomer (4.2 g), mp 118-121°C. A solution of this product in 8N HCl (87 mL) and glacial acetic acid (22 mL) was heated at reflux overnight. The solution was concentrated on a rotary evaporator, and the residue taken up in H₂O and extracted with ether. The aqueous layer was concentrated to dryness to give a 1:1 mixture of 3(S)-ethyl-2(S)-proline hydrochloride and α -methylbenzylamine.

3(S)-Ethyl-2(S)-proline containing α -methylbenzylamine (2.0 g, 0.0128 mol) was dissolved in dioxane (10 mL) and H₂O (10 mL) with stirring and cooling to 0°C. N,N-diisopropylethylamine (2.2 mL, 0.0128 mol) and di-*tert*-butyl-dicarbonate (2.79 g, 0.0128 mol) were added and stirring was continued at 23°C for 48 h. The reaction mixture was partitioned between EtOAc (60 mL) and H₂O (30 mL), the organic layer washed with 0.5N NaOH (2 x 40 mL), the aqueous layers combined and washed with EtOAc (30 mL) and this layer back-extracted with 0.5 N NaOH (30 mL). The aqueous layers were combined and carefully acidified at 0°C with 1N HCl to pH 3. This mixture was extracted with EtOAc (3 x 40 mL), the organics combined, dried (MgSO₄), filtered and concentrated to give N-[(*tert*-Butyloxy)carbonyl]-3(S)-ethyl-2(S)-proline as a colorless oil. N-[(*tert*-Butyloxy)carbonyl]-3(S)-ethyl-2(S)-proline was dissolved in EtOAc (50 mL) and the solution was saturated with HCl gas with cooling in an ice-H₂O bath. The solution was stoppered and stirred at 0°C. for 3 hr. Argon was

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bubbled through the solution to remove excess HCl, and the solution was concentrated to dryness to give 3(S)-ethyl-2(S)-proline hydrochloride.

Step G: N-(t-Butyloxycarbonyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-proline

5 3(S)-Ethyl-2(S)-proline hydrochloride (2.33 g, 0.013 mol) was dissolved in CH₃OH (20 mL), treated with 3A molecular sieves (2 g) and KOAc (1.27 g, 0.013 mol) to adjust the pH of the reaction mixture to 4.5-5, then N-[(*tert*-Butyloxy)carbonyl-prolinal (Pettit et
10 al., J. Org. Chem. (1994) **59**, [21] 6287-95) (3.36 g, 0.017 mol) was added, and the mixture was stirred for 16 hrs at room temperature. The reaction mixture was filtered, quenched with aq satd NaHCO₃ (5 mL) and concentrated to dryness. The residue was extracted with CHCl₃. The extract was dried (MgSO₄), filtered, and concentrated to
15 give the title compound and inorganic salts.

Step H: N-(t-Butyloxycarbonyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester

20 N-(t-Butyloxycarbonyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-proline (2.4 g, 0.008 mol), methionine isopropyl ester hydrochloride (2.21 g, 0.0097 mol), HOBt (1.49 g, 0.0097 mol) and EDC (1.86 g, 0.0097 mol) were dissolved in DMF (15 mL) at room temperature and treated with N-methylmorpholine (3 mL, 0.024 mol).
25 The reaction mixture was stirred overnight at room temperature, then concentrated and partitioned between EtOAc and H₂O. The organic layer was washed with aq satd NaHCO₃ solution, brine, and dried (MgSO₄). The crude product was chromatographed on a flash silica gel column eluting with hexane: EtOAc, 7:3 to give N-(t-butyloxy-carbonyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine
30 isopropyl ester.

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isopropyl ester hydrochloride

N-(t-butyloxycarbonyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester (1.38 g, 0.0028 mol) was dissolved in EtOAc (40 mL), cooled to -20°C, saturated with HCl gas, and stirred at 0°C. for 1.25 hr, and room temperature for 0.25 hr. Concentration to dryness gave pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester hydrochloride.

Step J: Preparation of 1H-Imidazole-4- acetic acid methyl ester hydrochloride

A solution of 1H-imidazole-4-acetic acid hydrochloride (4.00g, 24.6 mmol) in methanol (100 ml) was saturated with gaseous hydrogen chloride. The resulting solution was allowed to stand at room temperature (RT) for 18hr. The solvent was evaporated in vacuo to afford the title compound as a white solid.

¹H NMR(CDCl₃, 400 MHz) δ 8.85(1H, s), 7.45(1H, s), 3.89(2H, s) and 3.75(3H, s) ppm.

Step K: Preparation of 1-(Triphenylmethyl)-1H-imidazol-4-ylacetic acid methyl ester

To a solution of 1H-Imidazole-4- acetic acid methyl ester hydrochloride (24.85g, 0.141mol) in dimethyl formamide (DMF) (115ml) was added triethylamine (57.2 ml, 0.412mol) and triphenylmethyl bromide(55.3g, 0.171mol) and the suspension was stirred for 24hr. After this time, the reaction mixture was diluted with ethyl acetate (EtOAc) (1 l) and water (350 ml). The organic phase was washed with sat. aq. NaHCO₃ (350 ml), dried (Na₂SO₄) and evaporated in vacuo. The residue was purified by flash chromatography (SiO₂, 0-100% ethyl acetate in hexanes; gradient elution) to provide the title compound as a white solid.

¹H NMR (CDCl₃, 400 MHz) δ 7.35(1H, s), 7.31(9H, m), 7.22(6H, m), 6.76(1H, s), 3.68(3H, s) and 3.60(2H, s) ppm.

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Step L: Preparation of [1-(4-Cyanobenzyl)-1H-imidazol-5-yl]acetic acid methyl ester

To a solution of 1-(Triphenylmethyl)-1H-imidazol-4-ylacetic acid methyl ester (8.00g, 20.9mmol) in acetonitrile (70 ml) was added bromo-p-toluenitrile (4.10g, 20.92 mmol) and heated at 55°C for 3 hr. After this time, the reaction was cooled to room temperature and the resulting imidazolium salt (white precipitate) was collected by filtration. The filtrate was heated at 55°C for 18hr. The reaction mixture was cooled to room temperature and evaporated in vacuo. To the residue was added EtOAc (70 ml) and the resulting white precipitate collected by filtration. The precipitated imidazolium salts were combined, suspended in methanol (100 ml) and heated to reflux for 30min. After this time, the solvent was removed in vacuo, the resulting residue was suspended in EtOAc (75ml) and the solid isolated by filtration and washed (EtOAc). The solid was treated with sat aq NaHCO₃ (300ml) and CH₂Cl₂ (300ml) and stirred at room temperature for 2 hr. The organic layer was separated, dried (MgSO₄) and evaporated in vacuo to afford the title compound as a white solid :
¹H NMR(CDCl₃, 400 MHz) δ 7.65(1H, d, J=8Hz), 7.53(1H, s), 7.15(1H, d, J=8Hz), 7.04(1H, s), 5.24(2H, s), 3.62(3H, s) and 3.45(2H, s) ppm.

Step M: Preparation of [1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetic acid

A solution of [1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetic acid methyl ester (4.44g, 17.4mmol) in THF (100ml) and 1 M lithium hydroxide (17.4 ml, 17.4 mmol) was stirred at RT for 18 hr. 1 M HCl (17.4 ml) was added and the THF was removed by evaporation in vacuo. The aqueous solution was lyophilized to afford the title compound containing lithium chloride as a white solid.
¹H NMR(CD₃OD, 400 MHz) δ 8.22(1H, s), 7.74(1H, d, J=8.4Hz), 7.36(1H, d, J=8.4Hz), 7.15(1H, s), 5.43(2H, s) and 3.49(2H, s) ppm.

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Step N: Preparation of N-[(1-(4-Cyanobenzyl)-1H-imidazol-5-yl)acetyl]pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester

- [1-(4-Cyanobenzyl)-1H-imidazol-5-yl]acetic acid • LiCl
5 (0.416 g, 1.47 mmol), pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine isopropyl ester hydrochloride (Step I) (0.63 g, 1.33 mmol), HOObt (0.239 g, 1.47 mmol), and EDC (0.281 g, 1.47 mmol) were dissolved in degassed DMF (20 mL) with stirring at room temperature. N-methylmorpholine (0.8 mL, 5.32 mmol) was added to achieve a pH
10 of 7, and stirring was continued overnight. The reaction mixture was concentrated to remove most of the DMF, and the residue was partitioned between EtOAc and aq satd NaHCO₃ solution. The aq layer was washed with EtOAc, the organics combined, washed with brine and dried (MgSO₄). Filtration and concentration to dryness gave the title
15 compound after chromatography on silica gel eluting with CH₂Cl₂:CH₃OH, 95:5.

Anal. calcd for C₃₃H₄₆N₆O₄S • 0.7 H₂O:

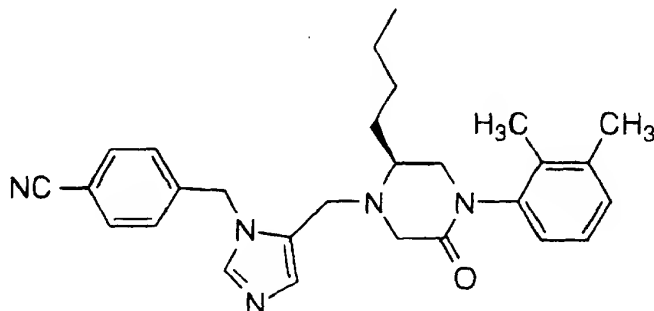
C, 62.38; H, 7.52; N, 13.23;

Found: C, 62.40; H, 7.17; N, 13.11.

20 FAB MS 623 (M+1)

EXAMPLE 5

- 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-
25 dimethylphenyl)piperazin-5-one



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1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-
2(S)-(2-methoxyethyl)piperazin-5-one ditrifluoroacetic acid salt

5 Step A: N-Methoxy-N-methyl 4-benzyloxy-2(S)-(tert-
 butoxycarbonylamino)butanamide
 4-Benzyloxy-2(S)-(tert-butoxycarbonylamino)butanoic acid
 (1.00 g, 3.23 mmol) was converted to the title compound following the
 procedure described in Example 24, Step A, using EDC · HCl (0.680 g,
10 3.55 mmol). HOBt (0.436 g, 3.23 mmol) and N,O-dimethylhydroxyl-
 amine hydrochloride (0.473 g, 4.85 mmol) in DMF (50 mL) at pH 7.
 After workup, the title compound was obtained as a clear gum.

15 Step B: 4-(1-Benzyloxyethyl)-2(S)-(tert-butoxycarbonylamino)
 butanal
 The title compound was obtained by lithium aluminum
 hydride reduction of the product of Step A using the procedure
 described in Example 24, Step B.

20 Step C: N-(2,3-Dimethylphenyl)-4-(2-benzyloxyethyl)-2-(S)-(tert-
 butoxycarbonylamino)butanamine
 The title compound was prepared from the product of Step
 C according to the procedure described in Example 24, Step C, using
 2,3-dimethylaniline (0.505 mL, 4.14 mmol), sodium triacetoxymor-
 phide (1.20 g, 5.65 mmol) and crushed molecular sieves (1 g) at pH
25 5 in dichloroethane (20 mL). The title compound was obtained after
 purification on silica gel, eluting with 15% ethyl acetate in hexane.

30 Step D: 2(S)-(2-Benzyloxyethyl)-1-tert-butoxycarbonyl-4-(2,3-
 dimethylphenyl)piperazin-5-one
 The title compound was prepared from the product of Step
 C according to the procedure described in Example 24, Step D, using
 chloroacetyl chloride (0.21 mL, 2.57 mmol) in 60 mL 1:1 ethyl acetate:
 saturated sodium bicarbonate, followed by reaction of the crude product
 with sodium hydride (0.373 g, 60% dispersion in oil, 9.32 mmol) in

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DMF (30 mL). After workup, the crude product was chromatographed on silica gel with 30% ethyl acetate in hexane to obtain the title compound.

5 Step E: 1-tert-Butoxycarbonyl-4-(2,3-dimethylphenyl)-2(S)-(2-hydroxyethyl)piperazin-5-one

The product from Step D was dissolved in methanol (40 mL) and 10% Pd/C was added (0.160 g). The reaction was shaken under 60 psi hydrogen overnight. The catalyst was removed by
10 filtration, and the solvent evaporated to give the title compound.

Step F: 1-tert-Butoxycarbonyl-4-(2,3-dimethylphenyl)-2(S)-(2-methoxyethyl)piperazin-5-one

The product from Step E (0.241 g, 0.688 mmol) was
15 dissolved in DMF (10 mL) containing methyl iodide (0.21 mL, 3.44 mmol) and the stirred solution cooled to 0°C under nitrogen. Sodium hydride (0.070 g, 60% dispersion in oil, 1.72 mmol) was added and the reaction stirred for 1h. The reaction was quenched with water, and the DMF removed under vacuum. The residue was partitioned between
20 ethyl acetate and water, and the organic phase washed with saturated brine and dried over magnesium sulfate. The crude product was chromatographed on silica gel with 40% ethyl acetate in hexane to give the title compound.

25 Step G: 4-(2,3-Dimethylphenyl)-2(S)-(2-methoxyethyl)-1-[4-(1-triphenylmethylimidazolyl)methyl]piperazin-5-one

The product from Step F (0.113 g, 0.312 mmol) was converted to the title compound according to the procedure described in Example 24, Step E, except using 30% trifluoroacetic acid in
30 dichloromethane (10 mL) for 1 h for the initial deprotection. The volatiles were removed *in vacuo*, and the residue dissolved in dichloroethane. Triethylamine was added to obtain pH 5. Sodium triacetoxyborohydride (0.100 g, 0.468 mmol) and 1-triphenylmethyl-

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4-imidazolylcarboxaldehyde (0.1164 g, 0.343 mmol) was added. The reaction was stirred overnight at 20°C then poured into saturated sodium bicarbonate solution. The organic phase was washed with saturated brine and dried over magnesium sulfate. Silica gel chromatography using 5% methanol in chloroform as eluant yielded the title compound.

Step H: 1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-2(S)-(2-methoxyethyl)piperazin-5-one
10 ditrifluoroacetic acid salt

The product from Step G (0.182 g, 0.312 mmol) was converted to the title compound according to the procedure described in Example 25, using 4-cyanobenzylbromide (0.061 g, 0.312 mmol) in acetonitrile (10 mL), followed by reaction of the crude imidazolium salt with triethylsilane (0.13 mL) and trifluoroacetic acid (2 mL) in dichloromethane (6 mL). Purification was accomplished by reverse phase preparative HPLC with a mixed gradient of 0%-70% acetonitrile/0.1% TFA; 100%-30% 0.1% aqueous TFA over 60 min. The title compound was isolated after lyophilization from water. FAB
20 ms (m+1) 458.

Anal. Calc. for $C_{27}H_{31}N_5O_2 \cdot 0.35 H_2O \cdot 2.0 TFA$:

C, 53.81; H, 4.91; N, 10.21.

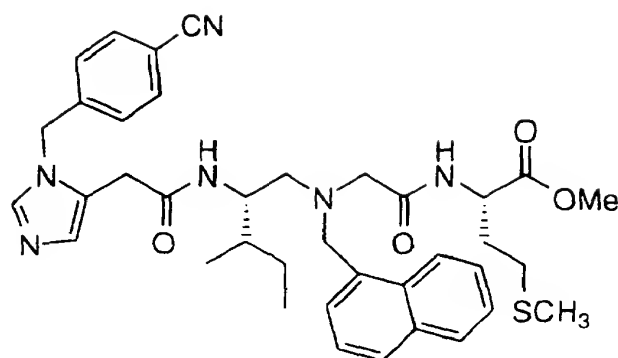
Found: C, 53.83; H, 4.95; N, 10.29.

25

EXAMPLE 6

N-[2(S)-N'-(1-(4-Cyanophenyl-methyl)-1H-imidazol-5-ylacetyl)amino-3(S)-methylpentyl]-N-1-naphthylmethyl-glycyl-methionine methyl ester

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Preparation of N-[2(S)-N'-(1-(4-Cyanophenylmethyl)-1H-imidazol-5-ylacetyl)amino-3(S)-methylpentyl]-N-1-naphthylmethyl-glycyl-methionine bis trifluoroacetate

5

Step A: Preparation of 1-(Triphenylmethyl)-1H-imidazol-4-ylacetic acid methyl ester (**23**)

To a suspension of 1H-imidazole-4-acetic acid methyl ester hydrochloride (**1**, 7.48, 42.4 mmol) in methylene chloride (200 ml) was added triethylamine (17.7 ml, 127 mmol) and triphenylmethyl bromide (16.4 g, 50.8 mmol) and stirred for 72 h. After this time, reaction mixture was washed with sat. aq. sodium bicarbonate (100 ml) and water (100 ml). The organic layer was evaporated *in vacuo* and purified by flash chromatography (30-100% ethyl acetate/hexanes gradient elution) to provide **23** as a white solid.

15

¹H NMR (CDCl₃, 400 MHz) δ 7.35 (1H, s), 7.31 (9H, m), 7.22 (6H, m), 6.76 (1H, s), 3.68 (3H, s) and 3.60 (2H, s) ppm.

Step B: Preparation of 1-(4-Nitrophenylmethyl)-1H-imidazol-5-ylacetic acid methyl ester (**16**)

20

To a solution of 1-(triphenylmethyl)-1H-imidazol-4-ylacetic acid methyl ester (**23**, 274 mg, 0.736 mmol) in acetonitrile (10 ml) was added 4-nitrobenzylbromide (159 mg, 0.736 mmol) and heated to 55°C for 16 h. After this time, the reaction was cooled to room temperature, treated with ethyl acetate (20 ml) and the resulting precipitate was filtered. The filtrate was concentrated to dryness *in vacuo* and the residue was redissolved in acetonitrile (4 ml) and heated to 65°C for

25

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3 h. After this time, the reaction mixture was evaporated to dryness and combined with initial precipitate. This residue was dissolved in methanol (5 ml) and heated to reflux for 30 min. The resulting solution was evaporated in vacuo and the residue was purified by flash chromatography (2-5% methanol/methylene chloride gradient elution) to provide **16**.

¹H NMR (CDCl₃, 400 MHz) δ 8.20 (2H, d, J=8.8 Hz), 7.53 (1H, s), 7.19 (2H, d, J=8.8 Hz), 7.03 (1H, s), 5.28 (2H, s), 3.61 (3H, s) and 3.44 (2H, s) ppm.

10

Step C: 1-(4-Nitrophenylmethyl)-1H-imidazol-5-ylacetic acid hydrochloride

1-(4-Nitrophenylmethyl)-1H-imidazol-5-ylacetic acid methyl ester (0.115 g, 0.42 mmol) was dissolved in 1.0N hydrochloric acid (10 ml) and heated at -55°C for 3 h. The solution was evaporated *in vacuo* to give the compound as a white solid.

¹H NMR (CD₃OD, 400 MHz) δ 9.06 (1H, s), 8.27 (2H, d, J=8.8 Hz), 7.61 (1H, s), 7.55 (2H, d, J=8.8 Hz), 5.63 (2H, s) and 3.81 (2H, s) ppm.

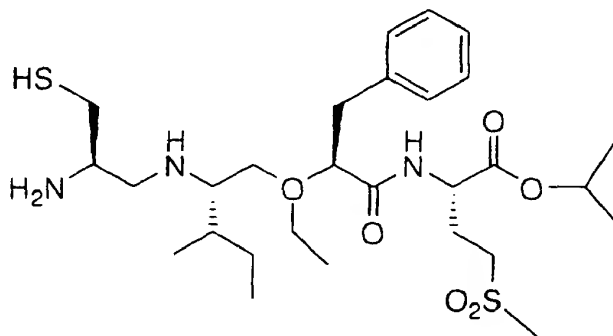
20 Step D: N-[2(S)-N'-(1-(4-Nitrophenylmethyl)-1H-imidazol-5-ylacetyl)amino-3(S)-methylpentyl]-N-1-naphthylmethyl-glycyl-methionine methyl ester bis trifluoroacetate

To a solution of 1-(4-nitrophenylmethyl)-1H-imidazol-5-ylacetic acid hydrochloride, N-[2(S)-amino-3(S)-methylpentyl]-N-naphthylmethyl-glycyl-methionine methyl ester bis hydrochloride (209 mg, 0.392 mmol) and 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HOBT, 64 mg, 0.39 mmol) in methylene chloride (10 ml) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 75.2 mg, 0.392 mmol) and triethylamine (219 µl, 1.57 mmol) and the mixture stirred overnight at room temperature. After this time, satd. aq. sodium bicarbonate (10 ml) was added and the mixture was extracted with methylene chloride. The combined extracts were washed with satd. aq. sodium bicarbonate (10 ml) and the solvent evaporated *in vacuo*.

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EXAMPLE 7

5 2(S)-[2(S)-[2(R)-Amino-3-mercapto]propylamino-3(S)-methyl]-
pentyloxy-3-phenylpropionyl-methionine sulfone isopropyl ester



10 The title compound is prepared in accordance with WO
94/10138 published on May 11, 1994, incorporated by reference.

BIOLOGICAL ASSAYS.

The ability of compounds of the present invention to inhibit cancer can be demonstrated using the following assays.

15

Raf kinase assay

Raf kinase activity *in vitro* is measured by the phosphorylation of its physiological substrate MEK (Map/ERK kinase). Phosphorylated MEK is subsequently trapped on a filter membrane and
20 incorporation of radio-labeled phosphate is quantitated by scintillation counting.

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MATERIALS

Activated Raf

Produced in Sf9 insect cells coinfecting with three
5 different baculoviruses expressing epitope-tagged Raf, and the upstream
activators Val¹²-H-Ras, and Lck. The epitope sequence Glu-Tyr-Met-
Pro-Met-Glu ("Glu-Glu") was fused to the carboxy-terminus of full-
length c-Raf.

10 MEK

Catalytically inactive MEK is produced in Sf9 cells infected
with baculovirus expressing epitope-tagged MEK with a lysine⁹⁷ to
alanine mutation (K97A). The epitope sequence Glu-Tyr-Met-Pro-Met-
15 Glu ("Glu-Glu") was fused to the amino-terminus of full-length MEK1.

Anti "Glu-Glu" antibody

A hybridoma cell line expressing an antibody specific for
the "Glu-Glu" epitope was obtained from Gernot Walter, UCSD. Cells
were grown and antibodies were purified as described (Grussenmeyer
20 et al., Proc. Natl. Acad. Sci. U.S.A., 82, pp. 7952-7954, 1985).

Column buffer

20 mM Tris, pH 8, 100 mM NaCl, 1 mM EDTA, 2.5 mM
EGTA, 10 mM MgCl₂, 2 mM DTT, 0.4 mM AEBSF, 0.1% n-octyl
25 glucopyranoside, 1 nM okadaic acid, and 10 µg/ml each of benzamidine,
leupeptin, pepstatin, and aprotinin (all SIGMA).

5x reaction buffer

125 mM HEPES pH=8.0, 25 mM MgCl₂, 5 mM EDTA, 5
30 mM Na₃VO₄, 100 µg/ml BSA

Enzyme dilution buffer

25 mM HEPES pH=8.0, 1 mM EDTA, 1 mM Na₃VO₄, 400
µg/ml BSA.

35

Stop solution

100 mM EDTA, 80 mM sodium pyrophosphate.

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Filter plates

Millipore Multiscreen #SE3M078E3, Immobilon-P (PVDF).

5

METHOD**A. Protein purification**

1. Sf9 insect cells were infected with baculovirus and grown as described (Williams et al., Proc. Natl. Acad. Sci. U.S.A., 89, pp. 2922-2926, 1992).
2. All subsequent steps were performed on ice or at 4°C. Cells were pelleted and lysed by sonication in column buffer. Lysates were spun at 17,000x g for 20 min, followed by 0.22 µm filtration.
3. Epitope-tagged proteins were purified by chromatography over a GammaBind Plus (Pharmacia) affinity column to which "Glu-Glu" antibody had been coupled. Proteins were loaded on the column, followed by washes with two column volumes of column buffer, and eluted with 50 µg/ml of peptide antigen (Glu-Tyr-Met-Pro-Met-Glu) in column buffer.

B. Raf kinase assay

1. Add 10 µl of inhibitor or control in 10% DMSO to assay plate.
2. Add 30 µl of reaction mix containing 10 µl 5x reaction buffer and 0.5 µl 1mM ³³P-γ-ATP (20 µCi/ml), 0.5 µl MEK (2.5 mg/ml), 1 µl 50 mM β-mercaptoethanol.
3. Start reaction by addition of 10 µl enzyme dilution buffer containing 1 mM DTT and an empirically determined amount of activated Raf that produces linear incorporation kinetics over the reaction time course.
4. Mix and incubate at room temperature for 90 min.
5. Stop reaction by addition of 50 µl stop solution.
6. Prewet filter plate with 70% ethanol and rinse with water.

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7. Transfer 90 µl aliquots of stopped reaction to filter plate.
8. Aspirate and wash four times with 200 µl H₂O.
9. Add 50 µl scintillation cocktail, seal plate, and count in Packard TopCount scintillation counter.

5

Map Kinase Phosphorylation assay

Inhibition of Raf kinase activity in intact cells is measured by determining the phosphorylation state of Map Kinase in TPA-stimulated C-33a human epithelial cells. Phosphorylated Map Kinase is detected by "Western" blot using an anti-phospho-Map Kinase antibody.

10

Materials

C33a Human Epithelial Cells

The C33a cell line is obtained from the ATCC repository, catalog # H TB31, and is maintained in DMEM (Mediatech) + 10% fetal bovine serum + 1% penicillin/streptomycin (Gibco) according to the instructions provided.

15

Anti-phospho-MAP Kinase antibody

The rabbit polyclonal anti-phospho-MAP kinase antibody is obtained from New England Biolabs (Beverly, MA)

20

Secondary antibody

The anti-rabbit antibody-alkaline phosphatase conjugate is obtained from New England Biolabs

25

Acrylamide Gel

Ten percent *bis*-acrylamide electrophoresis gels were obtained from Novex.

30

Blocking Buffer

1x Phosphate-buffered saline, 0.1% Tween-20, 5% nonfat dry milk.

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Antibody dilution buffer

1x phosphate-buffered saline, 0.05% Tween-20, 5% bovine serum albumin

5 Alkaline phosphatase substrate

The chemiluminescent alkaline phosphatase substrate, CDP-Star™, is obtained from New England Biolabs.

Assay Buffer

10 0.1 M diethanolamine, 1 mM MgCl₂.

Method

15 1. C33a cells are grown to confluency in 24 well plates, then starved for 24 hr in DMEM + 0.5 % charcoal-stripped serum.

2. Compound to be tested, dissolved in DMSO at 1000x concentration, is added to each well.

20 3. One hour later, TPA (dissolved in DMSO at 1000x concentration) is added at a final concentration of 100 ng/ml.

25 4. Twenty minutes later, the media is removed from all wells, and 100 µl of boiling hot reducing Laemmli sample buffer is added to each well. The plate is agitated, and the cell lysate is transferred to a 1.5 ml plastic microcentrifuge tube. Each lysate is then sonicated for 10 s, and placed in a boiling water bath for 5-10 minutes. Fifteen microliters of each sample is then loaded on a 10 % Laemmli polyacrylamide gel (Novex), and the gel electrophoresed according to the manufacturer's
30 instructions.

5. Proteins in the gel are electroblotted to a PVDF membrane, which is then rinsed in PBS and blocked with Blocking Buffer for approximately 1 hr at room temperature.

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6. The PVDF membrane is rinsed in PBS. The anti-phospho-MapK antibody, diluted approximately 1:500 in antibody dilution buffer, is incubated with the PVDF membrane with gentle agitation overnight at 4 °C.

7. The PVDF membrane is rinsed 3 times for 5 minutes with Blocking Buffer, then incubated with the secondary antibody, diluted approximately 1 : 1000 in antibody dilution buffer, for 1 hr with gentle agitation at room temperature.

8. The PVDF membrane is rinsed 5 times for 5 minutes with Blocking Buffer, then incubated with the chemiluminescent alkaline phosphatase substrate dissolved in Assay Buffer for approximately 5 minutes. The membrane is then rinsed, wrapped in plastic, and exposed to x-ray film to detect blotted proteins.

In the Raf kinase inhibition assay, the IC_{50} ranges from about 0.001 μ M to about 1.5 μ M.

In vitro inhibition of ras farnesyl transferase

Assays of farnesyl-protein transferase.

Partially purified bovine FPTase and Ras peptides (Ras-CVLS, Ras-CVIM and Ras-CAIL) were prepared as described by Schaber *et al.*, *J. Biol. Chem.* 265:14701-14704 (1990), Pompliano, *et al.*, *Biochemistry* 31:3800 (1992) and Gibbs *et al.*, *PNAS* U.S.A. 86:6630-6634 (1989), respectively. Bovine FPTase was assayed in a volume of 100 μ l containing 100 mM *N*-(2-hydroxy ethyl) piperazine-*N'*-(2-ethane sulfonic acid) (HEPES), pH 7.4, 5 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 100 mM [3H]-farnesyl diphosphate ([3H]-FPP; 740 CBq/mmol, New England Nuclear), 650 nM Ras-CVLS and 10 μ g/ml FPTase at 31°C for 60 min. Reactions were initiated with FPTase and stopped with 1 ml of 1.0 M HCL in ethanol. Precipitates were collected

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onto filter-mats using a TomTec Mach II cell harvester, washed with 100% ethanol, dried and counted in an LKB β -plate counter. The assay was linear with respect to both substrates, FPTase levels and time; less than 10% of the [^3H]-FPP was utilized during the reaction period.

5 Purified compounds were dissolved in 100% dimethyl sulfoxide (DMSO) and were diluted 20-fold into the assay. Percentage inhibition is measured by the amount of incorporation of radioactivity in the presence of the test compound when compared to the amount of incorporation in the absence of the test compound.

10 Human FPTase was prepared as described by Omer et al., Biochemistry 32:5167-5176 (1993). Human FPTase activity was assayed as described above with the exception that 0.1% (w/v) polyethylene glycol 20,000, 10 μM ZnCl_2 and 100 nM Ras-CVIM were added to the reaction mixture. Reactions were performed for 30 min.,
15 stopped with 100 μl of 30% (v/v) trichloroacetic acid (TCA) in ethanol and processed as described above for the bovine enzyme.

The farnesyl protein transferase inhibiting compounds are tested for inhibitory activity against human FPTase by the assay described above and the compounds can generally be found to have
20 IC_{50} of approximately 50 μM .

In vivo ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either Rat1 or NIH3T3 cells, which expressed viral Ha-ras p21. The
25 assay is performed essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991). Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours at 37°C, the cells are labelled in 3 ml methionine-free DMEM supplemented with 10% regular DMEM, 2% fetal bovine serum and 400
30 mCi [^{35}S]methionine (1000 Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1% NP40/20 mM HEPES, pH 7.5/5 mM MgCl_2 /1 mM DTT/10 mg/ml aprotinin/2 mg/ml leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation at

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100,000 x g for 45 min. Aliquots of lysates containing equal numbers of acid-precipitable counts are brought to 1 ml with IP buffer (lysis buffer lacking DTT) and immunoprecipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E. *et al.*, J. *Viro.* 43:294-304, (1982)). Following a 2 hour antibody incubation at 4°C, 200 ml of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG is added for 45 min. The immunoprecipitates are washed four times with IP buffer (20 mM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100/0.5% deoxycholate/0.1%/SDS/0.1 M NaCl) boiled in SDS-PAGE sample buffer and loaded on 13% acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in Enlightening, dried and autoradiographed. The intensities of the bands corresponding to farnesylated and nonfarnesylated ras proteins are compared to determine the percent inhibition of farnesyl transfer to protein.

15

In vivo growth inhibition assay

To determine the biological consequences of FPTase inhibition, the effect of the compounds of the instant invention on the anchorage-independent growth of Rat1 cells transformed with either a *v-ras*, *v-raf*, or *v-mos* oncogene is tested. Cells transformed by *v-Raf* and *v-Mos* maybe included in the analysis to evaluate the specificity of instant compounds for Ras-induced cell transformation.

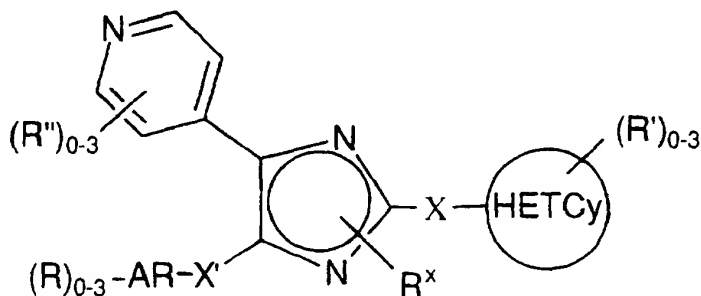
Rat 1 cells transformed with either *v-ras*, *v-raf*, or *v-mos* are seeded at a density of 1×10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay).

The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

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WHAT IS CLAIMED IS:

1. A method of treating cancer comprising administering to a mammalian patient in need of such treatment an effective amount of a RAF antagonist compound and an effective amount of a farnesyl protein transferase inhibiting compound.
2. A method of treating cancer in accordance with claim 1 wherein the cancer is selected from the group consisting of: cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung.
3. A method of treating cancer in accordance with claim 1 wherein the cancer is selected from the group consisting of: histiocytic lymphoma, lung adenocarcinoma and small cell lung cancers.
4. A method of treating cancer in accordance with claim 1 wherein the cancer is selected from the group consisting of: pancreatic and breast carcinoma.
5. A method of treating cancer in accordance with claim 1 wherein the RAF antagonist compound is selected from the group consisting of:
 - (a) a compound represented by formula (I-a):



(I-a)

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or a pharmaceutically acceptable salt thereof, wherein:

5 AR represents an aromatic group containing 6-10 atoms;

X and X' each independently represent $-(CH_2)_m-Y-(CH_2)_n-$,
 wherein m and n represent integers within the range of from 0 - 4, such
 that the sum of m and n is from 0 - 6; Y represents a member selected
 from the group consisting of: a direct bond; O; $S(O)_y$, with y equal to
 10 0, 1 or 2; $NR^{q'}$, with $R^{q'}$ as defined below; $C(O)$; $OC(O)$; $C(O)O$;
 $SO_xNR^{q'}$ with x equal to 1 or 2 and $R^{q'}$ as defined below; $NR^{q'}SO_x$;
 $C(O)NR^{q'}$ and $NR^{q'}C(O)$;



represents a 4 to 10 membered non-aromatic
 heterocycle containing at least one N atom, and optionally containing
 1-2 additional N atoms and 0-1 O or S atom;

15 R^x represents H, C_{1-6} alkyl(R^q)₃, OC_{1-6} alkyl(R^q)₃ or
 $C(O)C_{1-6}$ alkyl(R^q)₃;

each R and R" independently represents a member selected
 from the group consisting of: halo; hydroxy; C_{1-6} alkyl(R^q)₃;
 20 OC_{1-6} alkyl(R^q)₃; C_{3-8} cycloalkyl(R^q)₃; CN; $CONH_2$; $CONHC_{1-6}$
 alkyl(R^q)₃; $CON(C_{1-6}$ alkyl(R^q)₃)₂; NH_2 ; NHC_{1-6} alkyl(R^q)₃;
 $N(C_{1-6}$ alkyl(R^q)₃)₂; CO_2H ; CO_2C_{1-6} alkyl(R^q)₃; $C(O)C_{1-6}$
 alkyl(R^q)₃; aryl(R^q)₃; heteroaryl(R^q)₃; CF_3 ; SH; NO_2 ; SO_yC_{1-6}
 alkyl(R^q)₃, with y as defined above; SO_2NH_2 ; SO_2NHC_{1-6} alkyl(R^q)₃;
 25 $SO_2N(C_{1-6}$ alkyl(R^q)₃)₂; $NHSO_2C_{1-6}$ alkyl(R^q)₃, $NHSO_2$ aryl(R^q)₃,
 $NHSO_2$ heteroaryl(R^q)₃, $N(R^{q'})C(O)C_{1-6}$ alkyl(R^q)₃; $NR^{q'}C(O)NH(C_{1-6}$
 alkyl(R^q)₃); C_{2-4} alkenyl(R^q)₂₋₃ and C_{2-4} alkynyl(R^q)₁₋₃;

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each R' independently represents a member selected from the group consisting of: CONH₂; CONHC₁₋₆ alkyl(R^q)₃; CON(C₁₋₆ alkyl(R^q)₃)₂; CONHC₃₋₈ cycloalkyl(R^q)₃; CON(C₃₋₈ cycloalkyl(R^q)₃)₂; CO₂H; CO₂C₁₋₆ alkyl(R^q)₃; C(O)C₁₋₆ alkyl(R^q)₃; CO₂C₃₋₈ cycloalkyl(R^q)₃; C(O)C₃₋₈ cycloalkyl(R^q)₃; -[C(O)(CH₂)_j-CR⁵R⁶-(CH₂)_k-NR⁷]_p-R⁸; -C(O)C₃₋₈ cycloalkyl(R^q)₃; -C(O)heterocyclyl(R^q)₃; CON[C₁₋₆ alkyl(R^q)₃][C₃₋₈ cycloalkyl(R^q)₃]; C(O)aryl(R^q)₃; C(O)heteroaryl(R^q)₃;

10

wherein p represents 1, 2 or 3;

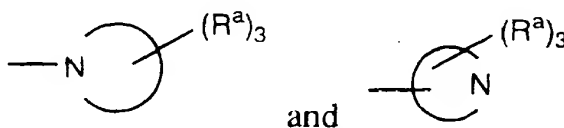
j and k are integers independently selected from 0 - 3;

each R⁵ and R⁶ independently represents H, aryl, C₁₋₆ alkyl(R^q)₃, or each CR⁵R⁶ taken in combination represents a 3, 4, 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or a heteroaryl group, wherein when p equals 1, at least one of j and k is 1, 2 or 3;

each R⁷ and R⁸ independently represents H, C₁₋₆ alkyl or aryl;

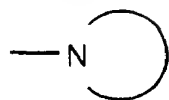
R^q represents a member selected from the group consisting of: R^{q'}; CN; CO₂H; CO₂C₁₋₄ alkyl; C(O)C₁₋₄ alkyl; aryl(R^a)₃; NH₂; NHC₁₋₆ alkyl(R^a)₃; N(C₁₋₆ alkyl(R^a)₃)₂; heteroaryl(R^a)₃; CONH₂; SH; S(O)_y C₁₋₆ alkyl(R^a)₃; C(O)NHC₁₋₆ alkyl(R^a)₃; C(O)N(C₁₋₆ alkyl(R^a)₃)₂; -heteroalkyl(R^a)₃; -NHC(O)NH₂; -NHC(NH)NH₂;

30

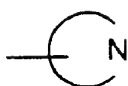


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wherein



and

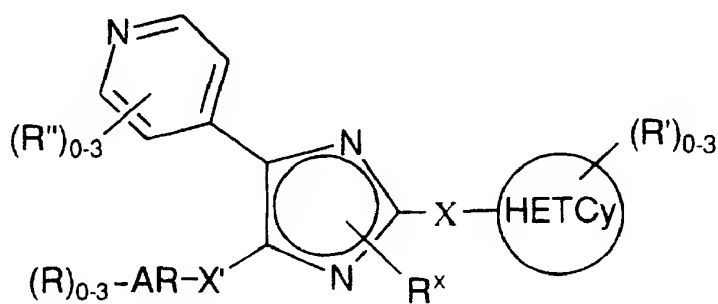


independently represent mono or bicyclic ring systems, non-aromatic or partially aromatic, containing from 5-10 ring atoms, 1-4 of which are N and 0-1 of which are O or S(O)_y, with y equal to 0, 1 or 2, optionally containing 1-2 carbonyl groups;

each R^a independently represents a member selected from the group consisting of: H, C₁₋₆ alkyl, OC₁₋₆ alkyl, aralkyl, substituted aralkyl, heteroaralkyl, substituted heteroaralkyl, aralkoxy, substituted aralkoxy, halo, hydroxy, CN, CONH₂, CONHC₁₋₆ alkyl, CON(C₁₋₆ alkyl)₂, CO₂H, CO₂C₁₋₆ alkyl, C(O)C₁₋₆ alkyl, phenyl, CF₃, SH, NO₂, SO_yC₁₋₆ alkyl, with y as defined above; SO₂NH₂, SO₂NHC₁₋₆ alkyl, NHSO₂(substituted aryl), NHSO₂(substituted heteroaryl), NHSO₂C₁₋₆ alkyl, NHSO₂aryl, NHSO₂heteroaryl, NH₂, NHC₁₋₆ alkyl, N(C₁₋₆ alkyl)₂, NHC(O)C₁₋₆ alkyl, NHC(O)NH(C₁₋₆ alkyl), C₂₋₄ alkenyl and C₂₋₄ alkynyl;

and R^q represents H, OH, C₁₋₄ alkyl, -OC₁₋₄ alkyl, aryl or C(O)C₁₋₄ alkyl;

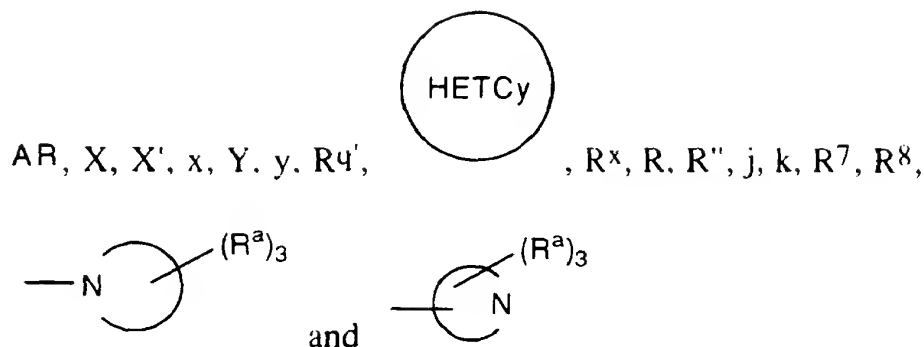
(b) a compound represented by formula (I-b):



(I-b)

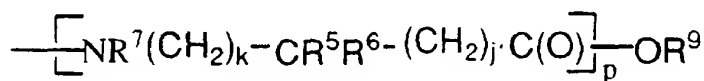
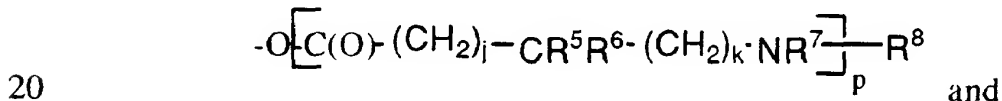
or a pharmaceutically acceptable salt thereof, wherein:

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5 are as defined above with respect to formula (I-a);

each R' independently represents a member selected from the group consisting of: hydroxy; C₁₋₆ alkyl(R^q)₃; C₃₋₈ cycloalkyl (R^q)₃; OC₁₋₆ alkyl(R^q)₃; OC₃₋₈ cycloalkyl(R^q)₃; heterocyclyl(R^q)₃;
 10 CN; NH(R^{q''}); NHC₁₋₆ alkyl(R^q)₃; N(C₁₋₆ alkyl(R^q)₃)₂; NHC₃₋₈ cycloalkyl(R^q)₃; N(C₃₋₈ cycloalkyl(R^q)₃)₂; CF₃; SH; NO₂; C₂₋₄ alkenyl(R^q)₂₋₃; aryl(R^q)₃; heteroaryl(R^q)₃; C₂₋₄ alkynyl(R^q)₁₋₃;
 -OC(O) C₃₋₈ cycloalkyl(R^q)₃; SO₂NH₂; SO₂NHC₁₋₆ alkyl(R^q)₃; SO₂N(C₁₋₆ alkyl(R^q)₃)₂; NHSO₂C₁₋₆alkyl(R^q)₃; NHSO₂aryl(R^q)₃;
 15 NHSO₂heteroaryl(R^q)₃; -OC(O)heterocyclyl(R^q)₃; N(R^{q'})C(O)C₁₋₆ alkyl(R^q)₃; NR^{q'}C(O)NH(C₁₋₆ alkyl(R^q)₃); -OC(O)C₁₋₆ alkyl(R^q)₃;
 -OC(O)aryl(R^q)₃; -OC(O)heteroaryl(R^q)₃; -C(=NR^{q'})NH₂; -C(=N^{q'})NHC₁₋₆ alkyl(R^q)₃; -C(=N^{q'})N(C₁₋₆ alkyl(R^q)₃)₂;



25 R⁵ and R⁶ are independently H, aryl, C₁₋₆ alkyl(R^q)₃, or CR⁵R⁶ in combination represents a 3, 4, 5 or 6 membered cycloalkyl or heterocyclyl group, an aryl group or a heteroaryl group;

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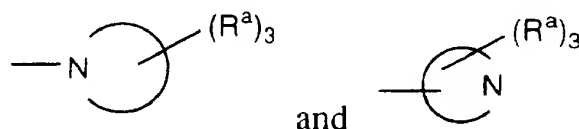
p represents 1, 2 or 3, with the proviso that when p represents 1, CR⁵R⁶ represents a 3, 4, 5 or 6 membered cycloalkyl group or a heterocyclyl group, an aryl group or a heteroaryl group, and at least one of j and k is 1, 2 or 3;

5

R⁹ represents H, a negative charge balanced by a positively charged group or a protecting group;

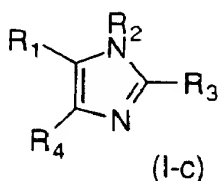
R⁹ represents a member selected from the group consisting of: R^q; CN; CO₂H; CO₂C₁₋₄ alkyl; C(O)C₁₋₄ alkyl; NH(R^q) ; aryl(R^a)₃; heteroaryl(R^a)₃; NHC₁₋₄ alkyl; N(C₁₋₄ alkyl)₂; CONH₂; SH; S(O)_y C₁₋₆ alkyl(R^a)₃; C(O)NHC₁₋₆ alkyl(R^a)₃; C(O)N(C₁₋₆ alkyl(R^a)₃)₂; NHC(NH)NH₂; -heteroalkyl(R^a)₃; -NHC(O)NH₂;

15



and R^q represents H, OH or OC₁₋₄ alkyl;

20 and (c) a compound represented by formula (I-c):



or a pharmaceutically acceptable salt thereof,
25 wherein:

R₁ is 4-pyridyl, pyrimidinyl, quinazolin-4-yl, quinolyl, isoquinolinyl, 1-imidazolyl or 1-benzimidazolyl which is optionally substituted with one or two substituents each of which is independently selected from C₁₋₄ alkyl, halogen, C₁₋₄ alkoxy, C₁₋₄ alkylthio, NR₁₀R₂₀, or N-heterocyclyl ring which ring has from 5 to 7 members and optionally

30

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contains an additional heteroatom selected from oxygen, sulfur or NR₂₂;

- R₂ is hydrogen, -(CR₁₀R₂₀)_n OR₁₂, heterocyclyl, heterocyclyl C₁-10 alkyl, C₁-10 alkyl, halo-substituted C₁-10 alkyl, C₂-10 alkenyl, C₂-10 alkynyl, C₃-7 cycloalkyl, C₃-7 cycloalkyl C₁-10 alkyl, C₅-7 cycloalkenyl, aryl, aryl C₁-10 alkyl, heteroaryl, heteroaryl C₁-10 alkyl, (CR₁₀R₂₀)_n'OR₁₃, (CR₁₀R₂₀)_n'S(O)_mR₂₅, (CR₁₀R₂₀)_n'NHS(O)₂R₂₅, (CR₁₀R₂₀)_n'NR₈R₉, (CR₁₀R₂₀)_n'NO₂, (CR₁₀R₂₀)_n'CN, (CR₁₀R₂₀)_n'S(O)_mNR₈R₉, (CR₁₀R₂₀)_n'C(Z)R₁₃, (CR₁₀R₂₀)_n'C(Z)OR₁₃, (CR₁₀R₂₀)_n'NR₁₀C(Z)NR₈R₉, (CR₁₀R₂₀)_n'C(Z)NR₁₃OR₁₂, (CR₁₀R₂₀)_n'NR₁₀C(Z)R₁₃, (CR₁₀R₂₀)_n'NR₁₀C(Z)NR₈R₉, (CR₁₀R₂₀)_n'N(OR₂₁)C(Z)NR₈R₉, (CR₁₀R₂₀)_n'N(OR₂₁)C(Z)R₁₃, (CR₁₀R₂₀)_n'C(=NOR₂₁)R₁₃, (CR₁₀R₂₀)_n'NR₁₀C(=NR₂₇)NR₈R₉, (CR₁₀R₂₀)_n'OC(Z)NR₈R₉, (CR₁₀R₂₀)_n'NR₁₀C(Z)NR₈R₉, (CR₁₀R₂₀)_n'C(Z)OR₁₀, 5-(R₂₅)-1,2,4-oxadiazol-3-yl or 4-(R₁₂)-5-(R₁₈R₁₉)-4,5-dihydro-1,2,4-oxadiazol-3-yl; wherein the aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocyclyl or heterocyclalkyl moieties may be optionally substituted;
- n' is an integer having a value of 1 to 10;
m is 0 or the integer 1 or 2;
R₃ is Q-(Y₁)_t;
Q is an aryl or heteroaryl group;
t is a number having a value of 1, 2 or 3;
- Z is oxygen or sulfur;
n is 0 or an integer from 1 to 10;
Y₁ is independently selected from hydrogen, C₁-5 alkyl, halo-substituted C₁-5 alkyl, halogen, or -(CR₁₀R₂₀)_nY₂;
Y₂ is -OR₈, -NO₂, -S(O)_mR₁₁, -SR₈, -S(O)_mOR₈, -S(O)_mNR₈R₉, -NR₈R₉, -O(CR₁₀R₂₀)_nNR₈R₉, -C(O)R₈, -CO₂R₈, -CO₂(CR₁₀R₂₀)_nCONR₈R₉, -ZC(O)R₈, -CN, -C(Z)NR₈R₉, NR-NR₁₀C(Z)R₈, -C(Z)NR₈OR₉, -NR₁₀C(Z)NR₈R₉, -NR₁₀S(O)_mR₁₁, -N(OR₂₁)C(Z)NR₈R₉, -N(OR₂₁)C(Z)R₈, -C(=NOR₂₁)R₈, -NR₁₀C(=NR₁₅)SR₁₁, -NR₁₀C(=NR₁₅)NR₈R₉,

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- NR₁₀C(=CR₁₄R₂₄)SR₁₁, -NR₁₀C(=CR₁₄R₂₄)NR₈R₉,
 -NR₁₀C(O)C(O)NR₈R₉, -NR₁₀C(O)C(O)OR₁₀,
 -C(=NR₁₃)NR₈R₉, -C(=NOR₁₃)NR₈R₉, -C(=NR₁₃)ZR₁₁,
 -OC(Z)NR₈R₉, -NR₁₀S(O)_mCF₃, -NR₁₀C(Z)OR₁₀, 5-(R₁₈)-
 1,2,4-oxadiazol-3-yl or 4-(R₁₂)-5-(R₁₈R₁₉)-4,5-dihydro-1,2,4-
 5 oxadiazol-3-yl;
 m' is a number having a value of 1 or 2;
 R₄ is phenyl, naphth-1-yl or naphth-2-yl which is optionally substituted
 by one or two substituents, each of which is independently selected,
 10 and which, for a 4-phenyl, 4-naphth-1-yl or 5-naphth-1-yl
 substituent, is halo, cyano, -C(Z)NR₇R₁₇, -C(Z)OR₂₃,
 -(CR₁₀R₂₀)_{m''}COR₃₆, SR₅, -SOR₅, OR₃₆, halo-substituted-C₁₋₄
 alkyl, C₁₋₄ alkyl, -ZC(Z)R₃₆, -NR₁₀C(Z)R₂₃ or
 -(CR₁₀R₂₀)_{m''}NR₁₀R₂₀ and which, for other positions of
 15 substitution, is halo, cyano, -C(Z)NR₁₆R₂₆, -C(Z)OR₈,
 -(CR₁₀R₂₀)_{m''}COR₈, -S(O)_mR₈, -OR₈, halo-substituted-C₁₋₄
 alkyl, C₁₋₄ alkyl, -(CR₁₀R₂₀)_{m''}NR₁₀C(Z)R₈, -NR₁₀S(O)_{m'}R₁₁,
 -NR₁₀S(O)_{m'}NR₇R₁₇, -ZC(Z)R₈ or -(CR₁₀R₂₀)_{m'}NR₁₆R₂₆;
 wherein m'' is 0 to 5 and m''' is 0 or 1;
 20 R₅ is hydrogen, C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl or NR₇R₁₇,
 excluding the moieties -SR₅ being -SNR₇R₁₇ and -SOR₅ being
 -SOH;
 R₆ is C₁₋₄ alkyl, halo-substituted-C₁₋₄ alkyl, C₁₋₄ alkenyl, C₂₋₄
 alkynyl or C₃₋₅ cycloalkyl;
 25 R₇ and R₁₇ are each independently selected from hydrogen or C₁₋₄
 alkyl, or R₇ and R₁₇ together with the nitrogen to which they are
 attached form a heterocyclic ring of 5 to 7 members which ring
 optionally contains an additional heteroatom selected from oxygen,
 sulfur or NR₂₂;
 30 R₈ is hydrogen, heterocyclyl, heterocyclalkyl or R₁₁;
 R₉ is hydrogen, C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇
 cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylalkyl, heteroaryl or
 heteroarylalkyl or R₈ and R₉ may together with the nitrogen to
 which they are attached form a heterocyclic ring of 5 to 7 members

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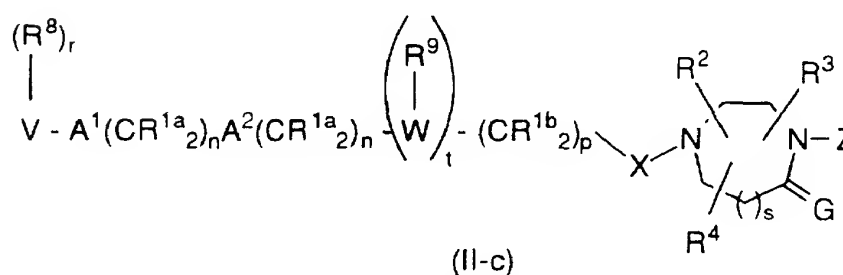
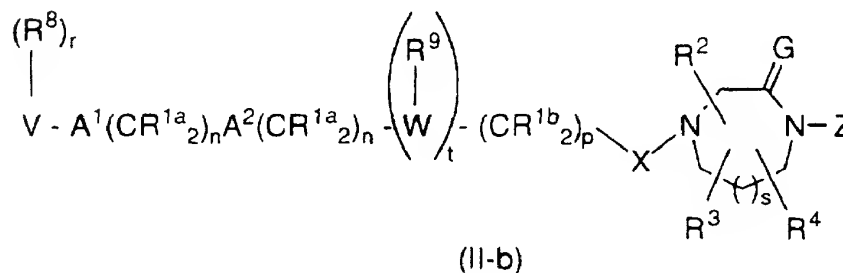
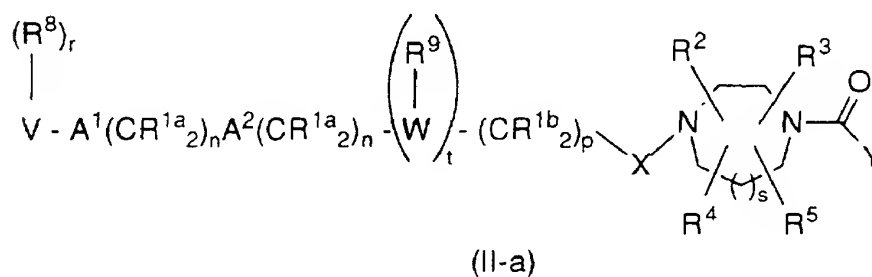
- which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₁₂;
- R₁₀ and R₂₀ are each independently selected from hydrogen and C₁₋₄ alkyl;
- 5 R₁₁ is C₁₋₁₀ alkyl, halo-substituted C₁₋₁₀ alkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₇ cycloalkyl, C₅₋₇ cycloalkenyl, aryl, arylalkyl, heteroaryl or heteroarylalkyl;
- R₁₂ is hydrogen, -C(Z)R₁₃ or optionally substituted C₁₋₄ alkyl, optionally substituted arylC₁₋₄ alkyl or S(O)₂R₂₅;
- 10 R₁₃ is hydrogen, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl, heterocyclyl C₁₋₁₀ alkyl, aryl, aryl C₁₋₁₀ alkyl, heteroaryl or heteroaryl C₁₋₁₀ alkyl;
- R₁₄ and R₂₄ is each independently selected from hydrogen, alkyl, nitro or cyano;
- 15 R₁₅ is hydrogen, cyano, C₁₋₄ alkyl, C₃₋₇ cycloalkyl or aryl;
- R₁₆ and R₂₆ is each independently selected from hydrogen or optionally substituted C₁₋₄ alkyl, optionally substituted aryl or optionally substituted aryl-C₁₋₄ alkyl, or together with the nitrogen which they are attached form a heterocyclic ring of 5 to 7 members
- 20 which ring optionally contains an additional heteroatom selected from oxygen, sulfur or NR₁₂;
- R₁₈ and R₁₉ is each independently selected from hydrogen, C₁₋₄ alkyl, substituted alkyl, optionally substituted aryl, optionally substituted arylalkyl or together denote a oxygen or sulfur;
- 25 R₂₁ is hydrogen, a pharmaceutically acceptable cation, C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, aryl, aryl C₁₋₄ alkyl, heteroaryl, heteroarylalkyl, heterocyclyl, aroyl, or C₁₋₁₀ alkanoyl;
- R₂₂ is R₁₀ or C(Z)-C₁₋₄ alkyl;
- R₂₃ is C₁₋₄ alkyl, halo-substituted-C₁₋₄ alkyl or C₃₋₅ cycloalkyl;
- 30 R₃₆ is hydrogen or R₂₃;
- R₂₅ is C₁₋₁₀ alkyl, C₃₋₇ cycloalkyl, heterocyclyl, aryl, arylalkyl, heterocyclyl, heterocyclyl-C₁₋₁₀ alkyl, heteroaryl or heteroarylalkyl;
- R₂₇ is hydrogen, cyano, C₁₋₄ alkyl, C₃₋₇ cycloalkyl or aryl.

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6. A method of treating cancer in accordance with claim 1 wherein the farnesyl transferase inhibiting compound is selected from the group consisting of:

5

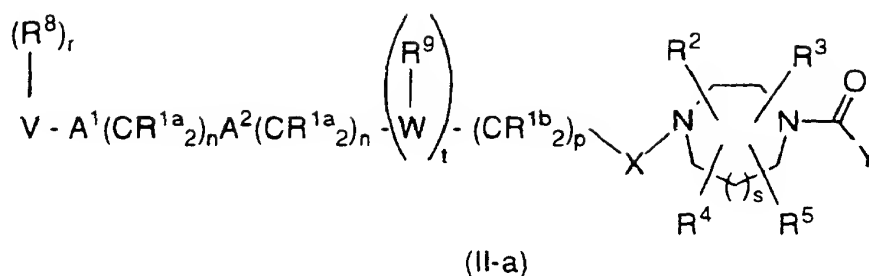
(a) a compound represented by one of formulas (II-a) through (II-c):



or a pharmaceutically acceptable salt thereof,

10 wherein with respect to formula (II-a):

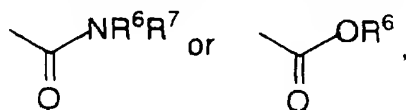
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R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl,
 5 C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN,
 NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃,
 $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl,
 heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆
 10 alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃,
 $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;

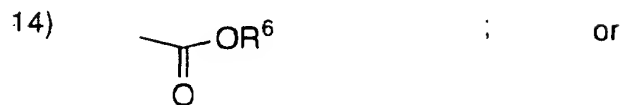
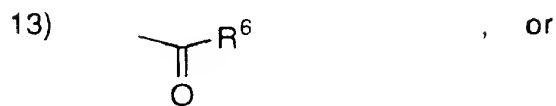
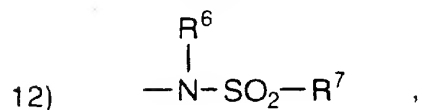
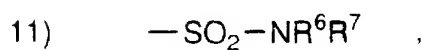
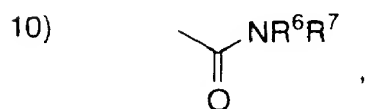
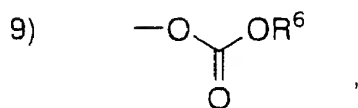
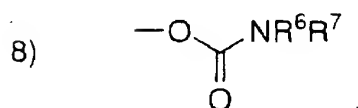
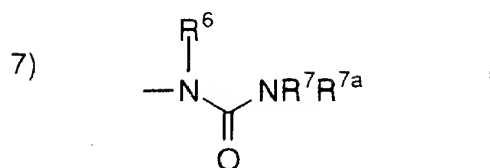
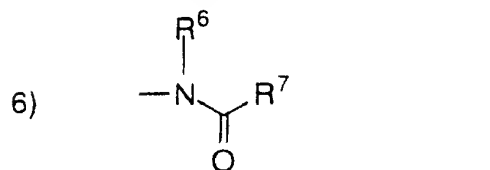
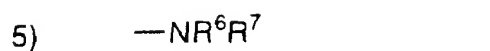
15 R^2 and R^3 are independently selected from: H; unsubstituted or substituted C₁-8 alkyl, unsubstituted or substituted C₂-8 alkenyl, unsubstituted or substituted C₂-8 alkynyl, unsubstituted or substituted aryl, unsubstituted or substituted heterocycle,



wherein the substituted group is substituted with one or more of:

- 20 1) aryl or heterocycle, unsubstituted or substituted with:
 - a) C₁-4 alkyl,
 - b) $(CH_2)_pOR^6$,
 - c) $(CH_2)_pNR^6R^7$,
 - d) halogen,
- 25 2) C₃-6 cycloalkyl,
- 3) OR^6 ,
- 4) SR^6 , $S(O)R^6$, SO_2R^6 ,

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5
10 R^2 and R^3 are attached to the same C atom and are combined to form $-(\text{CH}_2)_u-$ wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, $\text{S}(\text{O})_m$, $-\text{NC}(\text{O})-$, and $-\text{N}(\text{COR}^{10})-$;

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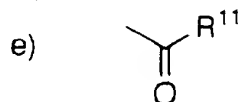
R^4 and R^5 are independently selected from H and CH_3 ;

- 5 and any two of R^2 , R^3 , R^4 and R^5 are optionally attached to the same carbon atom;

R^6 , R^7 and R^{7a} are independently selected from: H; C₁₋₄ alkyl, C₃₋₆ cycloalkyl, heterocycle, aryl, aroyl, heteroaroyl, arylsulfonyl,

- 10 heteroarylsulfonyl, unsubstituted or substituted with:

- a) C₁₋₄ alkoxy,
- b) aryl or heterocycle,
- c) halogen,
- d) HO,



- 15 f) $-SO_2R^{11}$, or
g) $N(R^{10})_2$; or

R^6 and R^7 may be joined in a ring;

R^7 and R^{7a} may be joined in a ring;

20

R^8 is independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C_{3-C10} cycloalkyl, C_{2-C6} alkenyl, C_{2-C6} alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$,
25 $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $R^{10}_2N-C(NR^{10})-$,
 $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or
 $R^{11}OC(O)NR^{10}-$, and
- c) C_{1-C6} alkyl unsubstituted or substituted by aryl,
heterocycle, C_{3-C10} cycloalkyl, C_{2-C6} alkenyl, C_{2-C6}
30 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$,

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$R^{10}C(O)NH-$, CN , $H_2N-C(NH)-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{10}OC(O)NH-$;

R^9 is selected from:

- 5 a) hydrogen,
- b) C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , NO_2 , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 10 c) C_1-C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

- 15 R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

- 20 A^1 and A^2 are independently selected from: a bond, $-CH=CH-$, $-C\equiv C-$, $-C(O)-$, $-C(O)NR^{10}-$, $-NR^{10}C(O)-$, O, $-N(R^{10})-$, $-S(O)_2N(R^{10})-$, $-N(R^{10})S(O)_2-$, or $S(O)_m$;

V is selected from:

- 25 a) hydrogen,
- b) heterocycle,
- c) aryl,
- d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N,
- 30 and
- e) C_2-C_{20} alkenyl,

provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

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W is a heterocycle;

X is $-\text{CH}_2-$, $-\text{C}(=\text{O})-$, or $-\text{S}(=\text{O})_m-$;

5 Y is aryl, heterocycle, unsubstituted or substituted with one or more of:

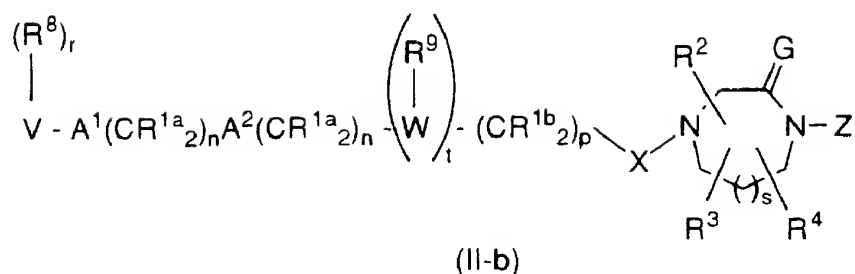
- 1) C_{1-4} alkyl, unsubstituted or substituted with:
 - a) C_{1-4} alkoxy,
 - b) NR^6R^7 ,
 - 10 c) C_{3-6} cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO ,
 - f) $-\text{S}(\text{O})_m\text{R}^6$, or
 - g) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$,
- 15 2) aryl or heterocycle,
- 3) halogen,
- 4) OR^6 ,
- 5) NR^6R^7 ,
- 6) CN ,
- 20 7) NO_2 ,
- 8) CF_3 ;
- 9) $-\text{S}(\text{O})_m\text{R}^6$,
- 10) $-\text{C}(\text{O})\text{NR}^6\text{R}^7$, or
- 11) $\text{C}_3\text{-C}_6$ cycloalkyl;

25

m is 0, 1 or 2;
 n is 0, 1, 2, 3 or 4;
 p is 0, 1, 2, 3 or 4;
 r is 0 to 5, provided that r is 0 when V is hydrogen;
 30 s is 0 or 1;
 t is 0 or 1; and
 u is 4 or 5;

with respect to formula (II-b):

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R^{1a} , R^{1b} , R^{10} , R^{11} , m , R^2 , R^3 , R^6 , R^7 , p , R^{7a} , u , R^8 , A^1 , A^2 , V , W , X , n , p , r , s , t and u are as defined above with respect to formula (II-a);

5 R^4 is selected from H and CH_3 ;

and any two of R^2 , R^3 and R^4 are optionally attached to the same carbon atom;

10 R^9 is selected from:

- a) hydrogen,
- b) alkenyl, alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 15 c) C_1 - C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

20

G is H_2 or O;

Z is aryl, heteroaryl, arylmethyl, heteroarylmethyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more of the following:

25

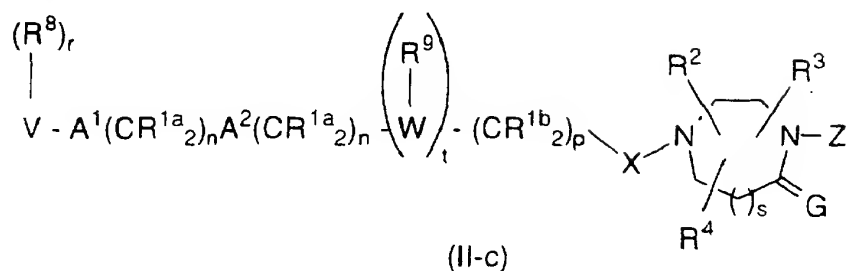
- 1) C_1 -4 alkyl, unsubstituted or substituted with:
 - a) C_1 -4 alkoxy,
 - b) NR^6R^7 ,
 - c) C_3 -6 cycloalkyl,

-246-

- d) aryl or heterocycle,
 e) HO,
 f) $-S(O)_mR^6$, or
 g) $-C(O)NR^6R^7$,
- 5 2) aryl or heterocycle,
 3) halogen,
 4) OR^6 ,
 5) NR^6R^7 ,
 6) CN,
 10 7) NO_2 ,
 8) CF_3 ;
 9) $-S(O)_mR^6$,
 10) $-C(O)NR^6R^7$, or
 11) C_3-C_6 cycloalkyl;

15

with respect to formula (II-c):



R^{1a} , R^{1b} , R^{10} , R^{11} , m , R^2 , R^3 , R^6 , R^7 , p , u , R^{7a} , R^8 , A^1 , A^2 , V , W , X ,
 n , r and t are as defined above with respect to formula (II-a);

20

R^4 is selected from H and CH_3 ;

and any two of R^2 , R^3 and R^4 are optionally attached to the same
 25 carbon atom;

G is O;

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Z is aryl, heteroaryl, arylmethyl, heteroarylmethyl, arylsulfonyl, heteroarylsulfonyl, unsubstituted or substituted with one or more of the following:

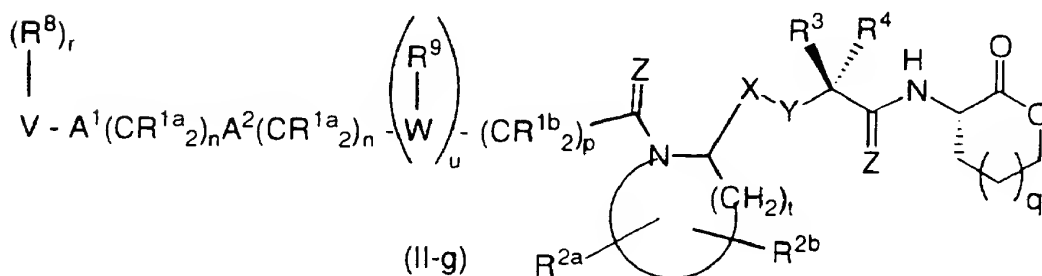
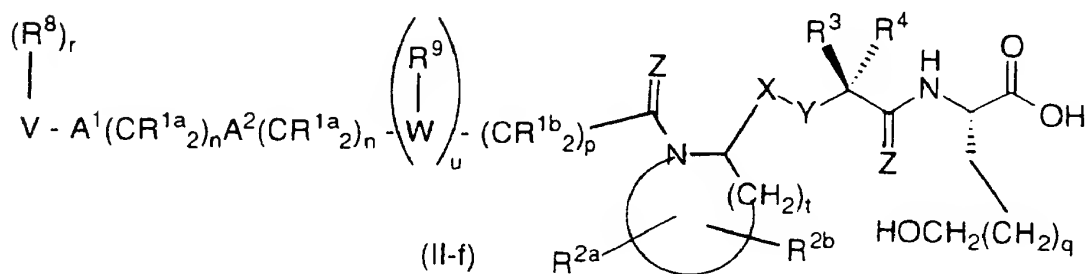
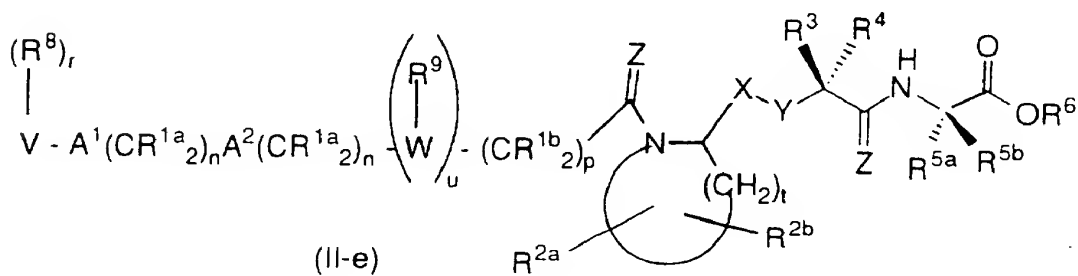
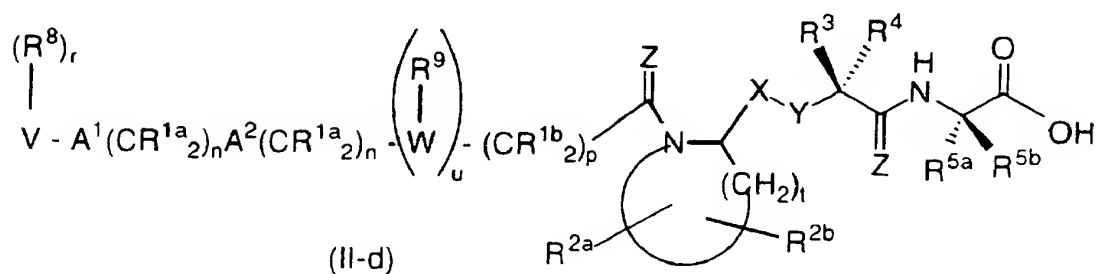
- 1) C₁₋₄ alkyl, unsubstituted or substituted with:
 - a) C₁₋₄ alkoxy,
 - b) NR⁶R⁷,
 - c) C₃₋₆ cycloalkyl,
 - d) aryl or heterocycle,
 - e) HO,
 - f) -S(O)_mR⁶, or
 - g) -C(O)NR⁶R⁷,
- 2) aryl or heterocycle,
- 3) halogen,
- 4) OR⁶,
- 5) NR⁶R⁷,
- 6) CN,
- 7) NO₂,
- 8) CF₃;
- 9) -S(O)_mR⁶,
- 10) -C(O)NR⁶R⁷, or
- 11) C₃₋₆ cycloalkyl;

and

s is 1;

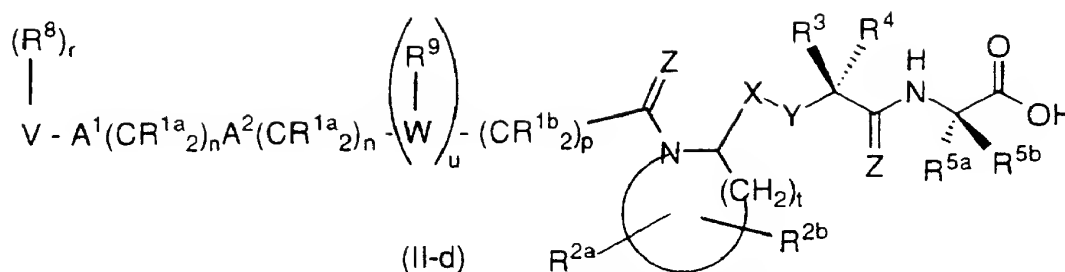
(b) a compound represented by formula (II-d) through (II-g):

-248-



5 wherein with respect to formula (II-d):

-249-



R¹¹, V, W, m, n, p and r are as defined above with respect to formula (II-a);

5

R^{1a} and R^{1b} are independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)-NR¹⁰-;

R^{2a} and R^{2b} are independently selected from:

- a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

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- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

5 R³ and R⁴ are independently selected from:

- a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 10 ii) methionine sulfone, and
 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
 wherein the substituent is selected from F, Cl, Br,
 N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,
 15 CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-,
 N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 and
 d) C₁-C₆ alkyl substituted with an unsubstituted or
 substituted group selected from aryl, heterocycle and
 20 C₃-C₁₀ cycloalkyl; or

R³ and R⁴ are combined to form - (CH₂)_s - ;

R^{5a} and R^{5b} are independently selected from:

- 25 a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 ii) methionine sulfone,
 30 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
 wherein the substituent is selected from F, Cl, Br,
 CF₃, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-,
 R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-,

-251-

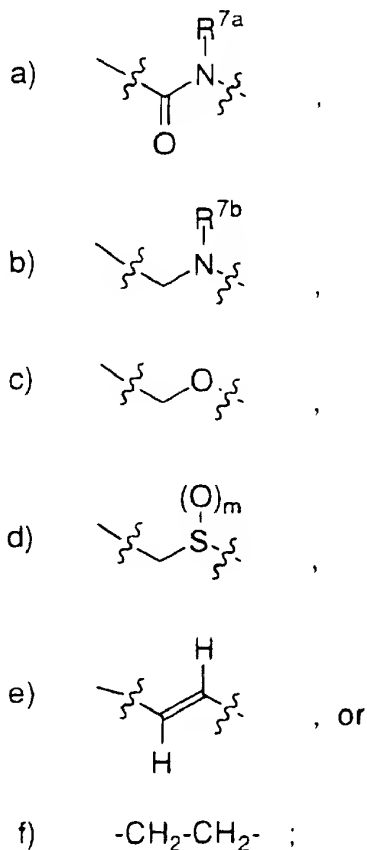
$R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and
 C_1-C_{20} alkyl,

- 5 d) C_1-C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3-C_{10} cycloalkyl; or

R^{5a} and R^{5b} are combined to form $-(CH_2)_s-$ wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, $S(O)_m$, $-NC(O)-$, and $-N(COR^{10})-$;

10

X-Y is



R^{7a} is selected from

- 15 a) hydrogen,

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- 5 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted
 or substituted group selected from aryl, heterocycle and
 C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- 10 a) hydrogen,
 b) unsubstituted or substituted aryl,
 c) unsubstituted or substituted heterocycle,
 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted
15 or substituted group selected from aryl, heterocycle and
 C₃-C₁₀ cycloalkyl,
 f) a carbonyl group which is bonded to an unsubstituted or
 substituted group selected from aryl, heterocycle, C₃-C₁₀
 cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an
20 unsubstituted or substituted group selected from aryl,
 heterocycle and C₃-C₁₀ cycloalkyl, and
 g) a sulfonyl group which is bonded to an unsubstituted or
 substituted group selected from aryl, heterocycle, C₃-C₁₀
 cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an
25 unsubstituted or substituted group selected from aryl,
 heterocycle and C₃-C₁₀ cycloalkyl;

R⁸ is independently selected from:

- 30 a) hydrogen,
 b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl,
 C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-,
 R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-,
 R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or
 R¹¹OC(O)NR¹⁰-, and

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- 5 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;

R⁹ is selected from:

- 10 a) hydrogen,
 b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
 15 c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

20 R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-, -N(R¹⁰)S(O)₂-, or S(O)_m;

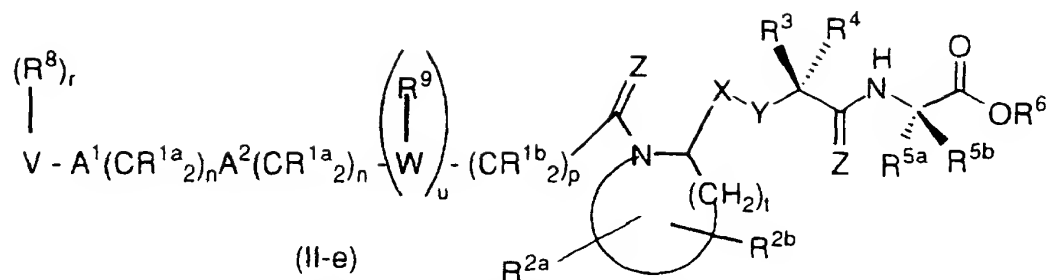
25 Z is independently H₂ or O;

s is 4 or 5;
 t is 3, 4 or 5; and
 u is 0 or 1;

30

with respect to formula (II-e):

-254-



R^{11} , W , m , n , p and r are as defined above with respect to formula (II-a);

- 5 R^{1a} and R^{1b} are independently selected from:
- a) hydrogen,
 - b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 - c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)-NR¹⁰-;
- R^{2a} and R^{2b} are independently selected from:
- a) hydrogen,
 - b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
 - c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

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- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;
- 5 R³ and R⁴ are independently selected from:
- a) a side chain of a naturally occurring amino acid,
 - b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - 10 ii) methionine sulfone,
 - c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,

15 CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl, and
 - d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and
 - 20 C₃-C₁₀ cycloalkyl; or

R³ and R⁴ are combined to form - (CH₂)_s - ;

- R^{5a} and R^{5b} are independently selected from:
- 25 a) a side chain of a naturally occurring amino acid,
 - b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone,
 - 30 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,

wherein the substituent is selected from F, Cl, Br, CF₃, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-,

-256-

$R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}$, and
 C_1 - C_{20} alkyl, and

- 5 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

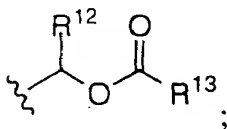
R^{5a} and R^{5b} are combined to form $-(CH_2)_s-$ wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, $S(O)_m$, $-NC(O)-$, and $-N(COR^{10})-$:

10

R^6 is

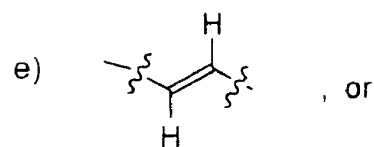
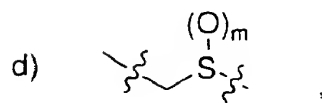
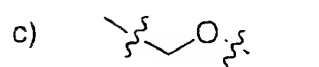
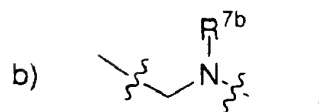
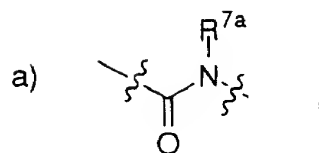
- a) substituted or unsubstituted C_1 - C_8 alkyl, substituted or unsubstituted C_5 - C_8 cycloalkyl, or substituted or unsubstituted cyclic amine, wherein the substituted alkyl, cycloalkyl or cyclic amine is substituted with 1 or 2 substituents independently selected from:
- 15 1) C_1 - C_6 alkyl,
 2) aryl,
 3) heterocycle,
 20 4) $-N(R^{11})_2$,
 5) $-OR^{10}$, or

b)



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X-Y is



5 R^{7a} is selected from

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
- 10 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- 15 a) hydrogen,
- b) unsubstituted or substituted aryl,

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- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
- f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
- g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

R⁸ is independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;

R⁹ is selected from:

- a) hydrogen,
- b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl,

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Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 ,
 $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
 $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and

- 5 c) C_1-C_6 alkyl unsubstituted or substituted by perfluoroalkyl.
 F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN,
 $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
 $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

10 R^{10} is independently selected from H, C_1-C_6 alkyl, benzyl, substituted
 aryl and C_1-C_6 alkyl substituted with substituted aryl;

R^{12} is hydrogen or C_1-C_6 alkyl;

15 R^{13} is C_1-C_6 alkyl;

A^1 and A^2 are independently selected from: a bond, $-CH=CH-$, $-C\equiv C-$,
 $-C(O)-$, $-C(O)NR^{10}-$, $-NR^{10}C(O)-$, O, $-N(R^{10})-$, $-S(O)_2N(R^{10})-$,
 $-N(R^{10})S(O)_2-$, or $S(O)_m$;

20 Z is independently H_2 or O;

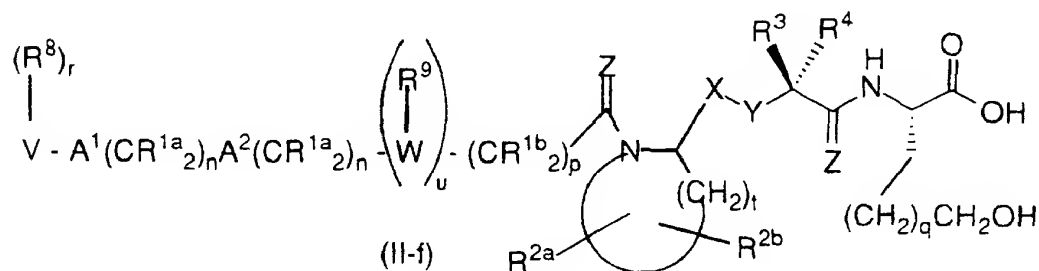
s is 4 or 5;

t is 3, 4 or 5; and

u is 0 or 1;

25

with respect to formula (II-f):



-260-

R^{11} , V, W, m, n, p and r are as defined above with respect to formula (II-a);

R^{1a} and R^{1b} are independently selected from:

- 5 a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}-$,
- 10 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocyclyl, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;
- 15 R^{2a} and R^{2b} are independently selected from:
 - a) hydrogen,
 - b) C₁-C₆ alkyl unsubstituted or substituted by C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N₃, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
 - 20 c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO₂, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N₃, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
 - 25 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

R^3 and R^4 are independently selected from:

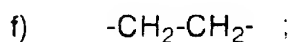
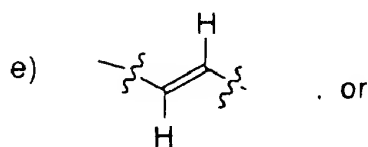
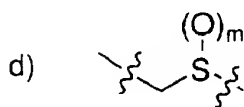
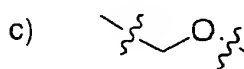
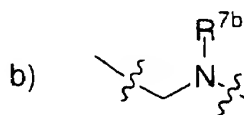
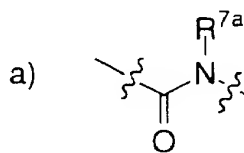
- 30 a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and

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- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
 wherein the substituent is selected from F, Cl, Br,
 N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-,
 5 CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-,
 N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 and
 d) C₁-C₆ alkyl substituted with an unsubstituted or
 substituted group selected from aryl, heterocycle and
 10 C₃-C₁₀ cycloalkyl; or

R³ and R⁴ are combined to form - (CH₂)_s - ;

X-Y is



15

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R^{7a} is selected from

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- 5 d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

10 R^{7b} is selected from

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- 15 e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
- f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
- 20 g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;
- 25

R⁸ is independently selected from:

- 30 a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-,

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- $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 5 c) C_1 - C_6 alkyl unsubstituted or substituted by aryl, heterocycle, C_3 - C_{10} cycloalkyl, C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NH-$, CN, $H_2N-C(NH)-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{10}OC(O)NH-$;

R^9 is selected from:

- 10 a) hydrogen.
- b) C_2 - C_6 alkenyl, C_2 - C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 , $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$, and
- 15 c) C_1 - C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, $(R^{10})_2N-C-(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$;

- 20 R^{10} is independently selected from H, C_1 - C_6 alkyl, benzyl, substituted aryl and C_1 - C_6 alkyl substituted with substituted aryl;

R^{12} is hydrogen or C_1 - C_6 alkyl;

- 25 R^{13} is C_1 - C_6 alkyl;

A^1 and A^2 are independently selected from: a bond, $-CH=CH-$, $-C\equiv C-$, $-C(O)-$, $-C(O)NR^{10}-$, $-NR^{10}C(O)-$, O, $-N(R^{10})-$, $-S(O)_2N(R^{10})-$, $-N(R^{10})S(O)_2-$, or $S(O)_m$;

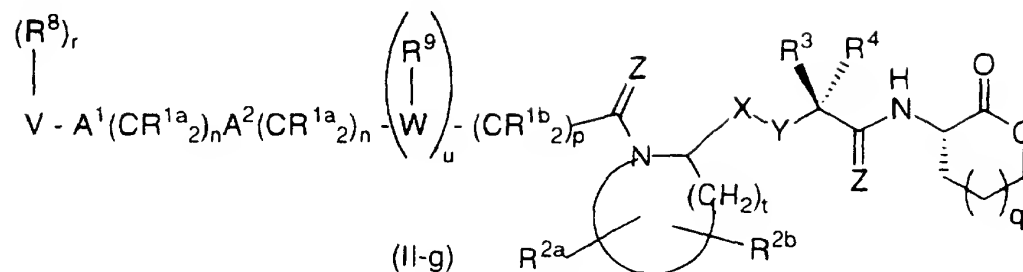
- 30 Z is independently H_2 or O;

q is 0, 1 or 2;
s is 4 or 5;

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t is 3, 4 or 5; and
u is 0 or 1;

with respect to formula (II-g):



R^{11} , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

R^{1a} and R^{1b} are independently selected from:

- 10 a) hydrogen,
b) aryl, heterocycle, C3-C10 cycloalkyl, C2-C6 alkenyl,
C2-C6 alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$,
CN, NO_2 ,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
15 $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
c) C1-C6 alkyl unsubstituted or substituted by aryl,
heterocycle, C3-C10 cycloalkyl, C2-C6 alkenyl, C2-C6
alkynyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
20 $-N(R^{10})_2$, or $R^{11}OC(O)-NR^{10}-$;

R^{2a} and R^{2b} are independently selected from:

- 25 - a) hydrogen,
b) C1-C6 alkyl unsubstituted or substituted by C2-C6
alkenyl, $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N_3 ,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or
 $R^{11}OC(O)NR^{10}-$,

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- c) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰), R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
- 5 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

R³ and R⁴ are independently selected from:

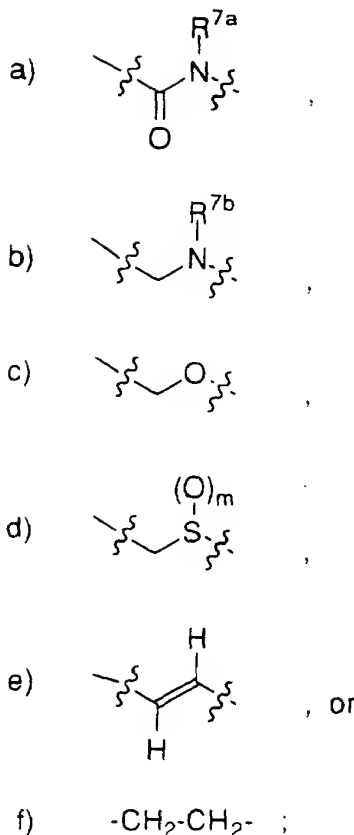
- 10 a) a side chain of a naturally occurring amino acid,
 b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 i) methionine sulfoxide, or
 ii) methionine sulfone,
- 15 c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl,
 20 and
 d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

25

R³ and R⁴ are combined to form - (CH₂)_s - ;

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X-Y is

R^{7a} is selected from

- a) hydrogen,
- 5 b) unsubstituted or substituted aryl,
- c) unsubstituted or substituted heterocycle,
- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl, and
- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and
- 10 C₃-C₁₀ cycloalkyl;

R^{7b} is selected from

- a) hydrogen,
- b) unsubstituted or substituted aryl,
- 15 c) unsubstituted or substituted heterocycle,

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- d) unsubstituted or substituted C₃-C₁₀ cycloalkyl,
- e) C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl,
- 5 f) a carbonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl, and
- 10 g) a sulfonyl group which is bonded to an unsubstituted or substituted group selected from aryl, heterocycle, C₃-C₁₀ cycloalkyl and C₁-C₆ alkyl substituted with hydrogen or an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl;

15

R⁸ is independently selected from:

- a) hydrogen,
- b) aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, R¹⁰₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- 20 c) C₁-C₆ alkyl unsubstituted or substituted by aryl, heterocycle, C₃-C₁₀ cycloalkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NH-, CN, H₂N-C(NH)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹⁰OC(O)NH-;
- 25

R⁹ is selected from:

- 30 a) hydrogen,
- b) C₂-C₆ alkenyl, C₂-C₆ alkynyl, perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C-(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and

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- c) C₁-C₆ alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-;

5

R¹⁰ is independently selected from H, C₁-C₆ alkyl, benzyl, substituted aryl and C₁-C₆ alkyl substituted with substituted aryl;

R¹² is hydrogen or C₁-C₆ alkyl;

10

R¹³ is C₁-C₆ alkyl;

A¹ and A² are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR¹⁰-, -NR¹⁰C(O)-, O, -N(R¹⁰)-, -S(O)₂N(R¹⁰)-,

15

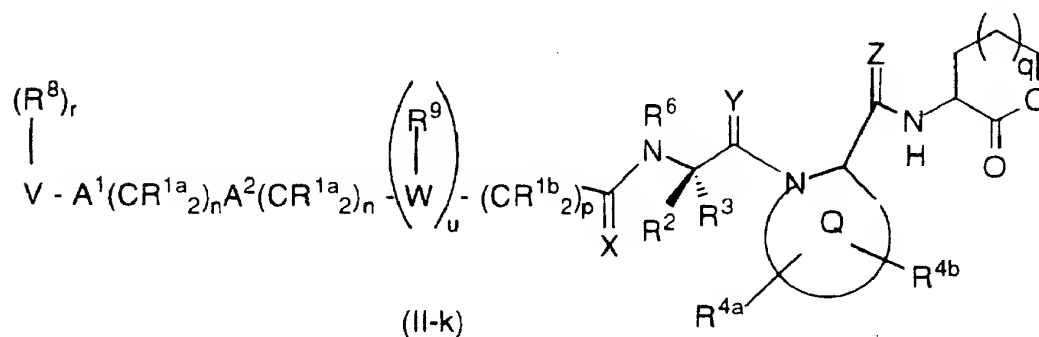
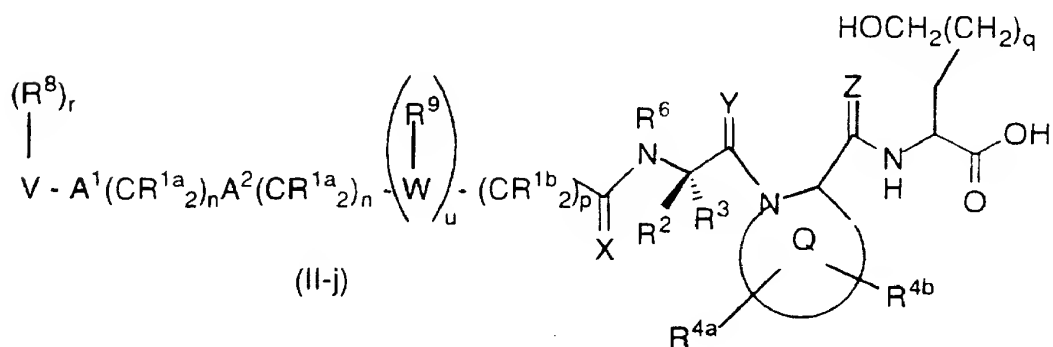
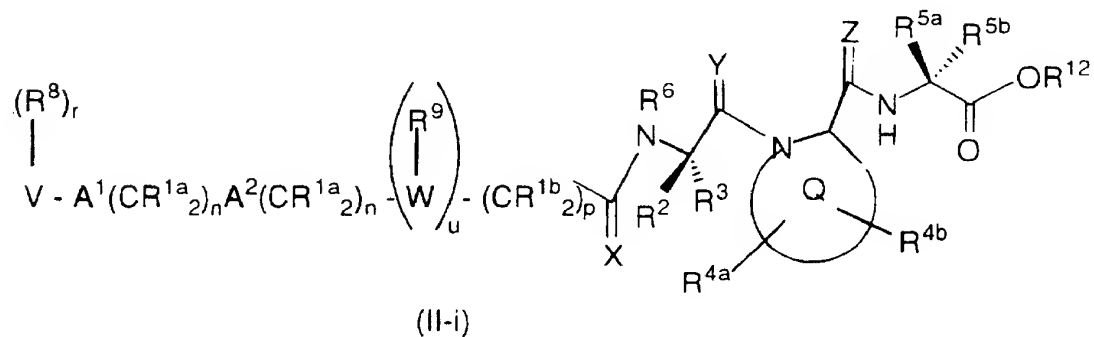
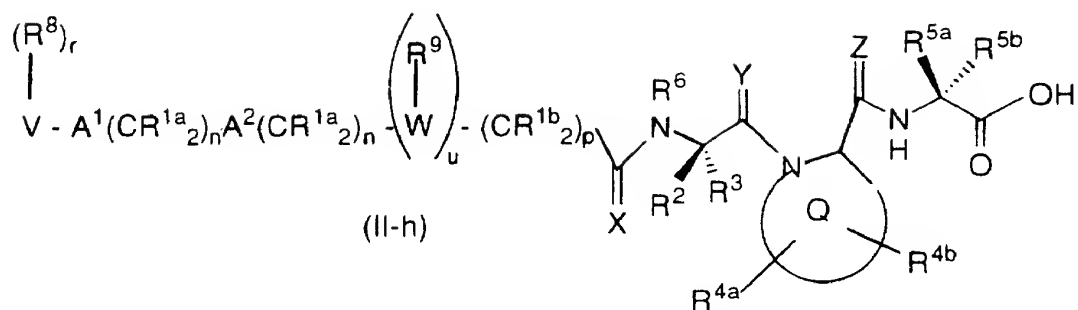
-N(R¹⁰)S(O)₂-, or S(O)_m;

Z is independently H₂ or O;

q is 0, 1 or 2;
 20 s is 4 or 5;
 t is 3, 4 or 5; and
 u is 0 or 1;

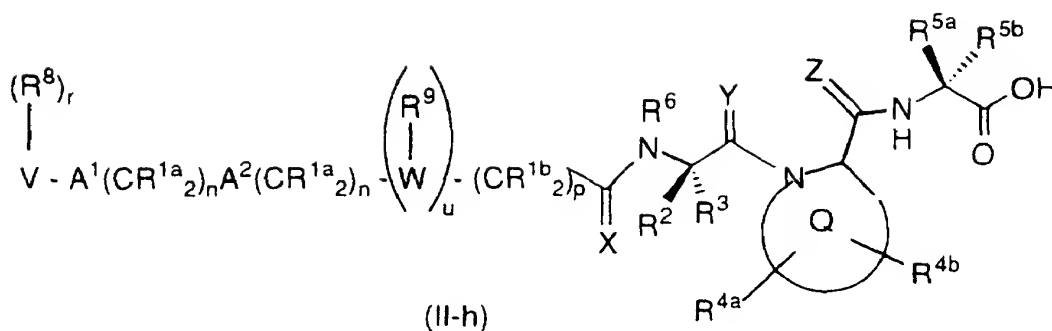
(c) a compound represented by one of formulas (II-h) through (II-k):

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or a pharmaceutically acceptable salt thereof,
wherein with respect to formula (II-h):

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5 R^{1a} , R^{1b} , R^8 , R^9 , R^{10} , R^{11} , A^1 , A^2 , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

R^2 and R^3 are independently selected from:

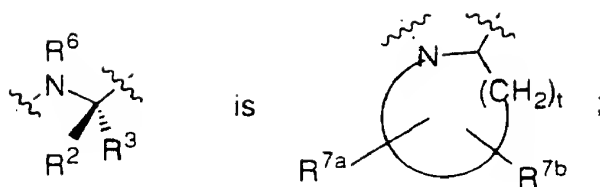
- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C_1 - C_{20} alkyl, C_2 - C_{20} alkenyl, C_3 - C_{10} cycloalkyl, aryl or heterocyclyl group,

15 wherein the substituent is selected from F, Cl, Br, $N(R^{10})_2$, NO_2 , $R^{10}O$ -, $R^{11}S(O)_m$ -, $R^{10}C(O)NR^{10}$ -, CN , $(R^{10})_2N-C(NR^{10})$ -, $R^{10}C(O)$ -, $R^{10}OC(O)$ -, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}$ - and C_1 - C_{20} alkyl, and
- d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

25 R^2 and R^3 are combined to form $-(CH_2)_s-$; or

R^2 or R^3 are combined with R^6 to form a ring such that

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R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
- 5 b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂,
 10 (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

R^{5a} and R^{5b} are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 20 i) methionine sulfoxide, or
 ii) methionine sulfone,
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,
 25 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl.
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and
 30 C₃-C₁₀ cycloalkyl; or

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R5a and R5b are combined to form $-(CH_2)_s-$ wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)-;

5 R6 is independently selected from hydrogen or C₁-C₆ alkyl;

Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;

10

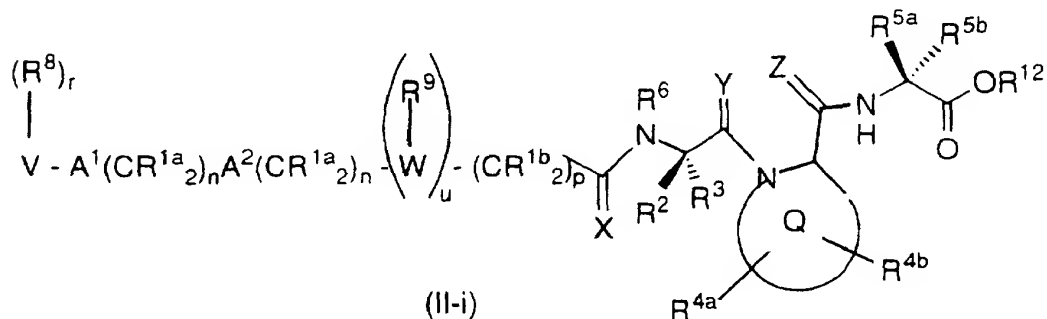
X, Y and Z are independently H₂ or O;

s is 4 or 5;

t is 3, 4 or 5; and

15 u is 0 or 1;

with respect to formula (II-i):



20

R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p and r are as previously defined with respect to formula (II-a);

R² and R³ are independently selected from:

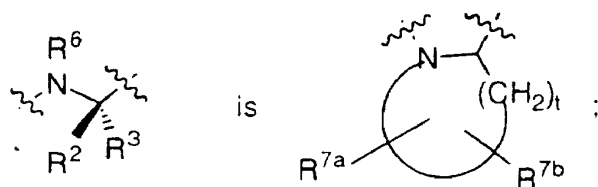
- 25 -
- a) a side chain of a naturally occurring amino acid,
 - b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or

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- ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,
 wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

R² and R³ are combined to form - (CH₂)_s - ; or

R² or R³ are combined with R⁶ to form a ring such that



R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C₃-C₁₀ cycloalkyl;

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R^{5a} and R^{5b} are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone,
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocycle group,

wherein the substituent is selected from F, Cl, Br, N(R¹⁰)₂, NO₂, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂, R¹¹OC(O)NR¹⁰- and C₁-C₂₀ alkyl.
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

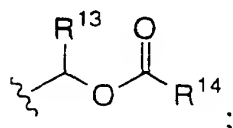
R^{5a} and R^{5b} are combined to form - (CH₂)_s - wherein one of the carbon atoms is optionally replaced by a moiety selected from: O, S(O)_m, -NC(O)-, and -N(COR¹⁰)-;

R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

R¹² is

- a) substituted or unsubstituted C₁-C₈ alkyl or substituted or unsubstituted C₅-C₈ cycloalkyl, wherein the substituent on the alkyl or cycloalkyl is selected from:
 - 1) aryl,
 - 2) heterocycle,
 - 3) -N(R¹¹)₂,
 - 4) -OR¹⁰, or

b)



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R^{13} is independently selected from hydrogen and C₁-C₆ alkyl;

R^{14} is independently selected from C₁-C₆ alkyl;

5

Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;

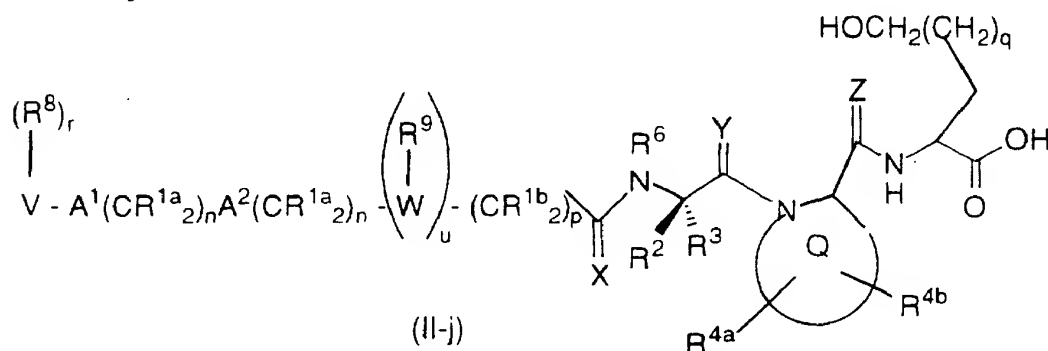
10 X, Y and Z are independently H₂ or O;

s is 4 or 5;

t is 3, 4 or 5; and

u is 0 or 1;

15 with respect to formula (II-j):



R^{1a} , R^{1b} , R^8 , R^9 , R^{10} , R^{11} , A^1 , A^2 , V , W , m , n , p and r are as previously defined with respect to formula (II-a);

20

R^2 and R^3 are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group.

25

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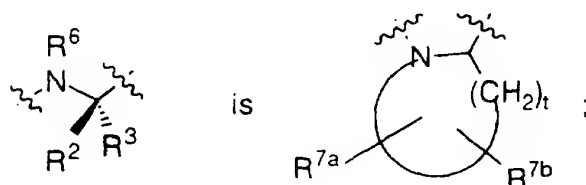
wherein the substituent is selected from F, Cl, Br,
 $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$,
 CN, $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$,
 N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1 - C_{20} alkyl,
 and

5

- d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C_3 - C_{10} cycloalkyl; or

10 R^2 and R^3 are combined to form $-(CH_2)_s-$; or

R^2 or R^3 are combined with R^6 to form a ring such that



15

R^{4a} , R^{4b} , R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
 b) C_1 - C_6 alkyl unsubstituted or substituted by alkenyl, $R^{10}O-$,
 $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, N_3 , $(R^{10})_2N-C(NR^{10})-$,
 20 $R^{10}C(O)-$, $R^{10}OC(O)-$, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}-$,
 c) aryl, heterocycle, cycloalkyl, alkenyl,
 $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN, NO_2 ,
 $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 ,
 $-N(R^{10})_2$ or $R^{11}OC(O)NR^{10}-$, and
 25 d) C_1 - C_6 alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and C_3 - C_{10} cycloalkyl;

25

R^6 is independently selected from hydrogen or C_1 - C_6 alkyl;

30

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Q is a substituted or unsubstituted nitrogen-containing C4-C9 mono or bicyclic ring system, wherein the non-nitrogen containing ring may be an aromatic ring, a C5-C7 saturated ring or a heterocycle;

5 X, Y and Z are independently H₂ or O;

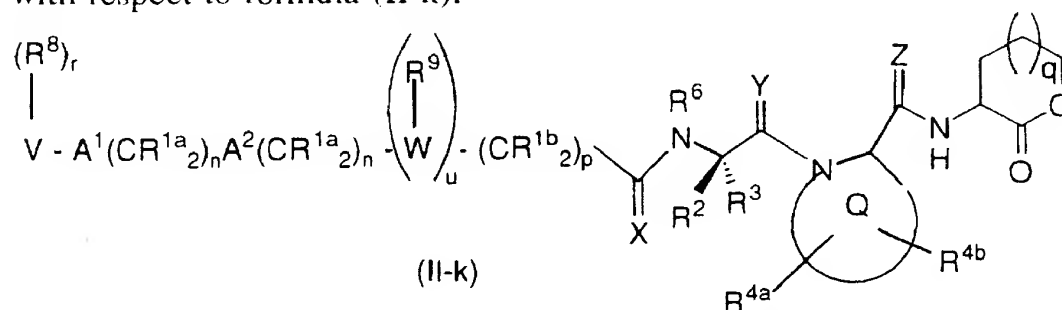
q is 0, 1 or 2;

s is 4 or 5;

t is 3, 4 or 5; and

10 u is 0 or 1;

with respect to formula (II-k):



15 R^{1a}, R^{1b}, R⁸, R⁹, R¹⁰, R¹¹, A¹, A², V, W, m, n, p, and r are as defined above with respect to formula (II-a);

R^2 and R^3 are independently selected from:

- a) a side chain of a naturally occurring amino acid,
- b) an oxidized form of a side chain of a naturally occurring amino acid which is:
 - i) methionine sulfoxide, or
 - ii) methionine sulfone, and
- c) substituted or unsubstituted C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₃-C₁₀ cycloalkyl, aryl or heterocyclyl group,

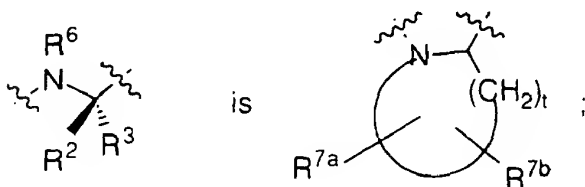
25 wherein the substituent is selected from F, Cl, Br, $N(R^{10})_2$, NO_2 , $R^{10}O-$, $R^{11}S(O)_m-$, $R^{10}C(O)NR^{10}-$, CN , $(R^{10})_2N-C(NR^{10})-$, $R^{10}C(O)-$, $R^{10}OC(O)-$, N_3 , $-N(R^{10})_2$, $R^{11}OC(O)NR^{10}-$ and C_1-C_{20} alkyl, and

-278-

- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocycle and C₃-C₁₀ cycloalkyl; or

5 R² and R³ are combined to form - (CH₂)_s - ; or

R² or R³ are combined with R⁶ to form a ring such that



10

R^{4a}, R^{4b}, R^{7a} and R^{7b} are independently selected from:

- a) hydrogen,
- b) C₁-C₆ alkyl unsubstituted or substituted by alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, N₃, (R¹⁰)₂N-C(NR¹⁰)-,
 15 R¹⁰C(O)-, R¹⁰OC(O)-, -N(R¹⁰)₂, or R¹¹OC(O)NR¹⁰-,
- c) aryl, heterocycle, cycloalkyl, alkenyl, R¹⁰O-, R¹¹S(O)_m-, R¹⁰C(O)NR¹⁰-, CN, NO₂, (R¹⁰)₂N-C(NR¹⁰)-, R¹⁰C(O)-, R¹⁰OC(O)-, N₃, -N(R¹⁰)₂ or R¹¹OC(O)NR¹⁰-, and
- d) C₁-C₆ alkyl substituted with an unsubstituted or substituted group selected from aryl, heterocyclyl and
 20 C₃-C₁₀ cycloalkyl;

R⁶ is independently selected from hydrogen or C₁-C₆ alkyl;

25

Q is a substituted or unsubstituted nitrogen-containing C₄-C₉ mono or bicyclic ring system, wherein the non-nitrogen containing ring may be
 - an aromatic ring, a C₅-C₇ saturated ring or a heterocycle;

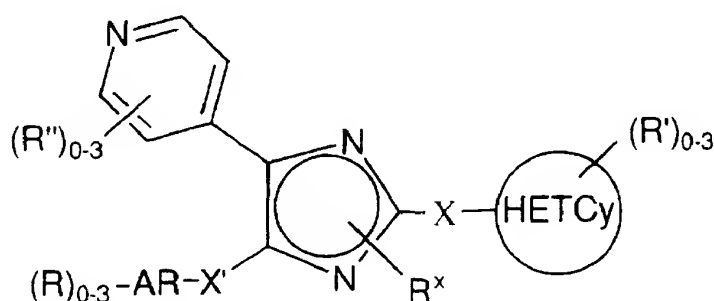
30 X, Y and Z are independently H₂ or O;

-279-

q is 0, 1 or 2;
 s is 4 or 5;
 t is 3, 4 or 5; and
 u is 0 or 1.

5

7. A method of treating cancer in accordance with claim 5 wherein the RAF antagonist is (a) a compound represented by formula (I-a):



10

(I-a)

selected from the group consisting of:

15 4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;

4-[4-fluorophenyl)-3-pyridin-yl-1H-imidazol-2-yl]-1-acetyl-piperidine;

20 3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;

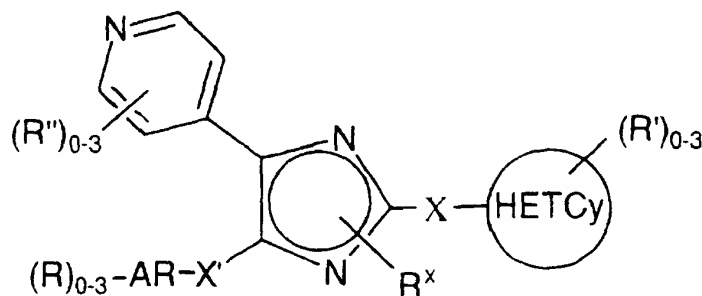
3-[4-fluorophenyl)-3-pyridin-yl-1H-imidazol-2-yl]-1-acetyl-piperidine;
 and

25 4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester,

-280-

or a pharmaceutically acceptable salt thereof.

8. A method of treating cancer in accordance with claim 5 wherein the RAF antagonist compound is (b) a compound represented by formula (I-b):



(I-b)

selected from the group consisting of:

10

4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;

4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

15

4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-benzyl-piperidine;

20

4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-ethyl-piperidine;

4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;

25

4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

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2-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-butyl)-isoindole-1,3-dione;

5 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-isoindole-1,3-dione;

2-(6-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-hexyl)-isoindole-1,3-dione;

10 4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-benzyl-piperidine;

15 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-2,3-dihydro-isoindol-1-one ditrifluoroacetic acid salt;

4-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-ethyl)-pyridine;

20 2-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-pentyl)-1,1-dioxobenzo[d]isothiazol-3-one;

25 2-(4-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-butyl)-1,1-dioxobenzo[d]isothiazol-3-one;

2-amino-1-{5-[4-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-ethanone dihydrochloride;

30 4-[5-(3-hydroxyphenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;

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3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidine:

3-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1-methyl-piperidine;

5

4-[5-(4-fluorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-1,4-dimethyl-piperidine;

10

4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine-1-carboxylic acid *tert*-butyl ester;

4-benzyl-[4-(4-fluorophenyl)-5-pyridin-4-yl-1H-imidazol-2-yl]-piperidine;

15

4-{5-(3,4-dichlorophenyl)-2-[1-(2-phenylethyl)-piperidin-4-yl]-1H-imidazol-4-yl}-pyridine;

4-{5-(3,4-dichlorophenyl)-2-[1-(3-phenylpropyl)-piperidin-4-yl]-1H-imidazol-4-yl}-pyridine;

20

2-(6-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-hexyl)-1,1-dioxobenzo[d]isothiazol-3-one;

25

2-(3-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-propyl)-1,1-dioxobenzo[d]isothiazol-3-one;

4-(5-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl-methyl}-imidazol-1-yl-methyl)-benzonitrile;

30

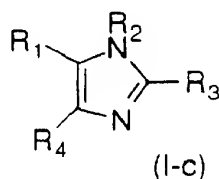
4-[2-[1-(4-benzyloxybenzyl)-piperidin-4-yl-5-(3,4-dichlorophenyl)-1H-imidazol-4-yl]-pyridine; and

2-(3-{4-[5-(3,4-dichlorophenyl)-4-pyridin-4-yl-1H-imidazol-2-yl]-piperidin-1-yl}-propyl)-isoindole-1,3-dione,

-283-

or a pharmaceutically acceptable salt thereof.

9. A method of treating cancer in accordance with claim
 5 wherein the RAF antagonist compound is (c) a compound represented
 by formula (I-c):



- 10 selected from the group consisting of:

4-[4-(4-fluorophenyl)-5-(4-pyridyl)imidazol-2-yl]benzamidoxime;

4-(1-naphthyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;

15

4-(1-naphthyl)-2-(4-methylthiophenyl)-5-(4-pyridyl)imidazole;

4-(2-naphthyl)-2-(4-methylthiophenyl)-5-(4-pyridyl)imidazole;

20 4-(2-naphthyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(3-thiophene)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(2-thiophene)-5-(4-pyridyl)imidazole;

25

4-(4-fluorophenyl)-2-(3-methylthiophenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(3-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;

30 4-(4-fluorophenyl)-2-(3-methylsulfonylphenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(2-methylthiophenyl)-5-(4-pyridyl)imidazole;

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4-(4-fluorophenyl)-2-(2-methylsulfinylphenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(2-methylsulfonylphenyl)-5-(4-pyridyl)imidazole;

5

4-(4-fluorophenyl)-2-(4-methoxyphenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1-methyl-5-(4-pyridyl)
imidazole;

10

4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-1-(N-
morpholinopropyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-2-(4-methylthiophenyl)-1-(N-morpholinopropyl)-5-
(4-pyridyl)imidazole;

15

4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-1-(N-morpholino-
propyl)-5-(4-pyridyl)imidazole;

20

4-(4-fluorophenyl)-1-(methylthio-1-propyl)-2-([4-N-
morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole;

4-(4-fluorophenyl)-1-(methylsulfinyl-1-propyl)-2-([4-N-
morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole; and

25

4-(4-fluorophenyl)-1-(methylsulfonyl-1-propyl)-2-([4-N-
morpholinomethyl]phenyl)-5-(4-pyridyl)imidazole,

or a pharmaceutically acceptable salt thereof.

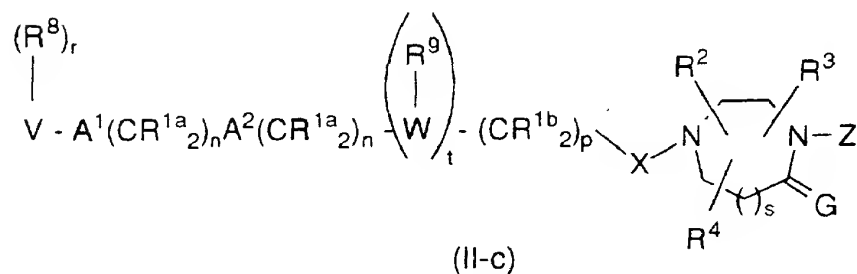
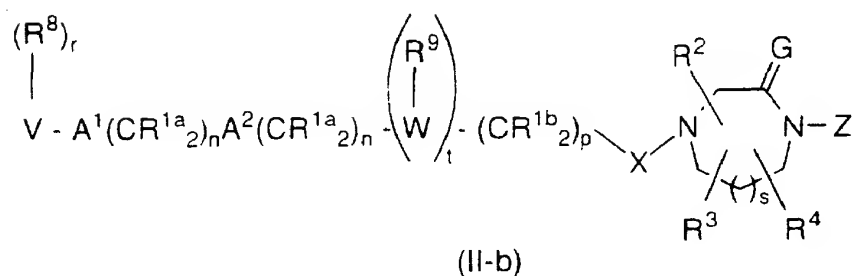
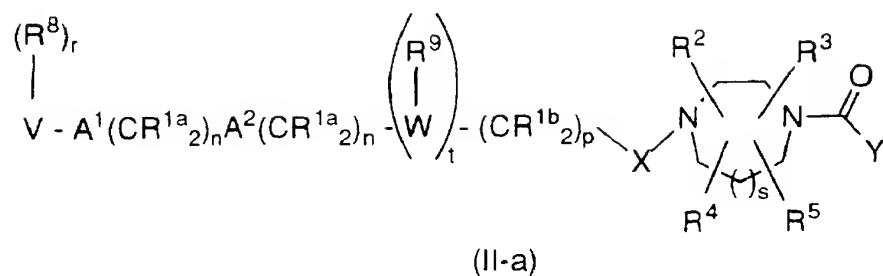
30

10. A method of treating cancer in accordance with claim
6 wherein the farnesyl transferase inhibiting compound is

(a) a compound represented by one of formulas (II-a) through (II-c):

35

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selected from the group consisting of:

2(S)-butyl-1-(2,3-diaminoprop-1-yl)-1-(1-naphthoyl)piperazine;

5

1-(3-amino-2-(2-naphthylmethylamino)prop-1-yl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

2(S)-butyl-1-[5-[1-(2-naphthylmethyl)]-4,5-dihydroimidazol]methyl-4-(1-naphthoyl)piperazine;

10

1-[5-(1-benzylimidazol)methyl]-2(S)-butyl-4-(1-naphthoyl)piperazine;

-286-

1 - { 5 - [1 - (4 - nitrobenzyl)] imidazolylmethyl } - 2 (S) - butyl - 4 - (1 - naphthoyl) piperazine;

5 1 - (3 - acetamidomethylthio - 2 (R) - aminoprop - 1 - yl) - 2 (S) - butyl - 4 - (1 - naphthoyl) piperazine;

2 (S) - butyl - 1 - [2 - (1 - imidazolyl) ethyl] sulfonyl - 4 - (1 - naphthoyl) piperazine;

10 2 (R) - butyl - 1 - imidazolyl - 4 - methyl - 4 - (1 - naphthoyl) piperazine;

2 (S) - butyl - 4 - (1 - naphthoyl) - 1 - (3 - pyridylmethyl) piperazine;

15 1 - 2 (S) - butyl - (2 (R) - (4 - nitrobenzyl) amino - 3 - hydroxypropyl) - 4 - (1 - naphthoyl) piperazine;

1 - (2 (R) - amino - 3 - hydroxyheptadecyl) - 2 (S) - butyl - 4 - (1 - naphthoyl) - piperazine;

20 2 (S) - benzyl - 1 - imidazolyl - 4 - methyl - 4 - (1 - naphthoyl) piperazine;

1 - (2 (R) - amino - 3 - (3 - benzylthio) propyl) - 2 (S) - butyl - 4 - (1 - naphthoyl) piperazine;

25 1 - (2 (R) - amino - 3 - [3 - (4 - nitrobenzylthio) propyl]) - 2 (S) - butyl - 4 - (1 - naphthoyl) piperazine;

2 (S) - butyl - 1 - [(4 - imidazolyl) ethyl] - 4 - (1 - naphthoyl) piperazine;

30 2 (S) - butyl - 1 - [(4 - imidazolyl) methyl] - 4 - (1 - naphthoyl) piperazine;

2 (S) - butyl - 1 - [(1 - naphth - 2 - ylmethyl) - 1 H - imidazol - 5 - yl] acetyl] - 4 - (1 - naphthoyl) piperazine;

-287-

2(S)-butyl-1-[(1-naphth-2-ylmethyl)-1H-imidazol-5-yl]ethyl]-4-(1-naphthoyl)piperazine;

1-(2(R)-amino-3-hydroxypropyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

5

1-(2(R)-amino-4-hydroxybutyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-(2-amino-3-(2-benzyloxyphenyl)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

10

1-(2-amino-3-(2-hydroxyphenyl)propyl)-2(S)-butyl-4-(1-naphthoyl)piperazine;

1-[3-(4-imidazolyl)propyl]-2(S)-butyl-4-(1-naphthoyl)-piperazine;

15

2(S)-*n*-butyl-4-(2,3-dimethylphenyl)-1-(4-imidazolylmethyl)-piperazin-5-one;

2(S)-*n*-butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)piperazin-5-one;

20

1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-dimethylphenyl)-2(S)-(2-methoxyethyl)piperazin-5-one;

2(S)-*n*-butyl-4-(1-naphthoyl)-1-[1-(1-naphthylmethyl)imidazol-5-ylmethyl]-piperazine;

25

2(S)-*n*-butyl-4-(1-naphthoyl)-1-[1-(2-naphthylmethyl)imidazol-5-ylmethyl]-piperazine;

30

2(S)-*n*-butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine;

-288-

2(S)-*n*-butyl-1-[1-(4-methoxybenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine;

5 2(S)-*n*-butyl-1-[1-(3-methyl-2-butenyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine;

2(S)-*n*-butyl-1-[1-(4-fluorobenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine;

10 2(S)-*n*-butyl-1-[1-(4-chlorobenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)piperazine;

1-[1-(4-bromobenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-naphthoyl)piperazine;

15 2(S)-*n*-butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethylbenzyl)imidazol-5-ylmethyl]-piperazine;

20 2(S)-*n*-butyl-1-[1-(4-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine;

2(S)-*n*-butyl-1-[1-(3-methylbenzyl)imidazol-5-ylmethyl]-4-(1-naphthoyl)-piperazine;

25 1-[1-(4-phenylbenzyl)imidazol-5-ylmethyl]-2(S)-*n*-butyl-4-(1-naphthoyl)-piperazine;

2(S)-*n*-butyl-4-(1-naphthoyl)-1-[1-(2-phenylethyl)imidazol-5-ylmethyl]-piperazine;

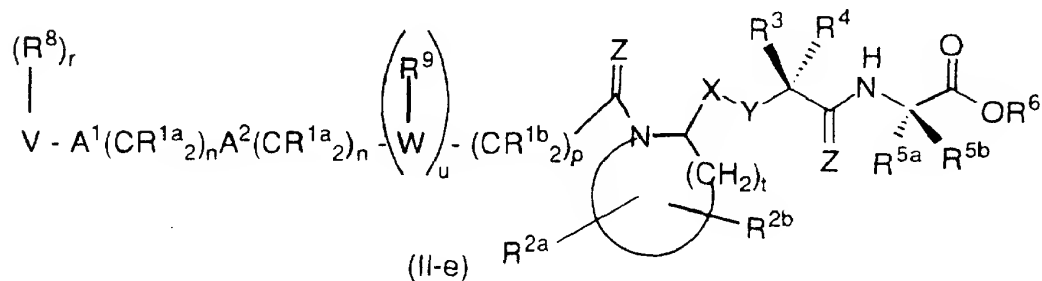
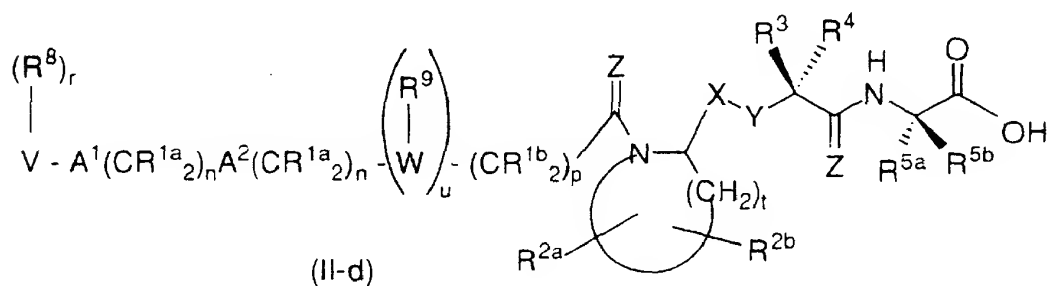
30 2(S)-*n*-butyl-4-(1-naphthoyl)-1-[1-(4-trifluoromethoxy)imidazol-5-ylmethyl]piperazine;

35 1-[[1-(4-cyanobenzyl)-1H-imidazol-5-yl]acetyl]-2(S)-*n*-butyl-4-(1-naphthoyl)piperazine;

-289-

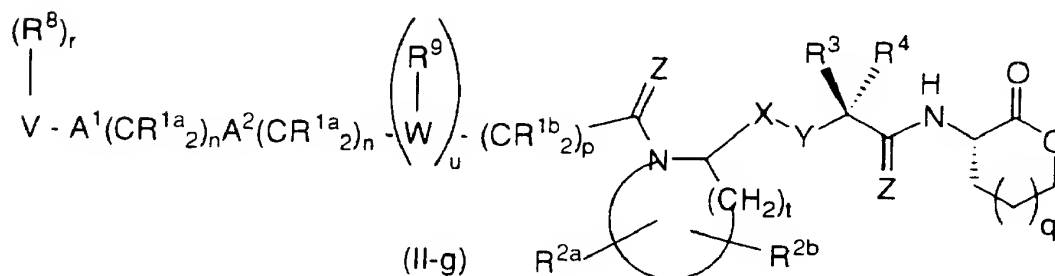
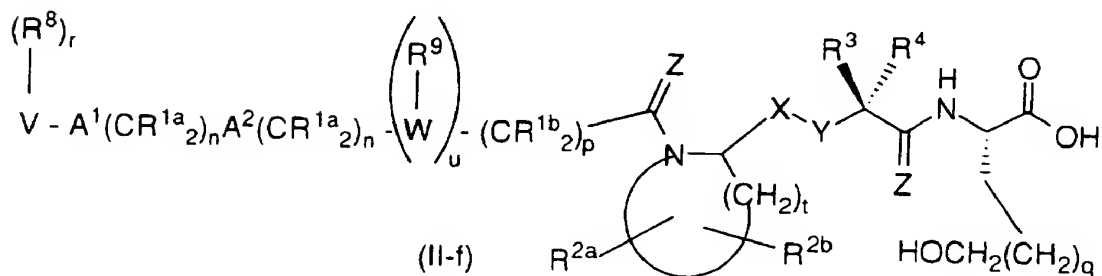
or a pharmaceutically acceptable salt thereof.

11. A method of treating cancer in accordance with claim
 5 6 wherein the farnesyl transferase inhibiting compound is (b) a
 compound represented by one of formulas (II-d) through (II-g):



10

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selected from the group consisting of:

- 5 N-[1-(4-imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycylmethionine
- N-[1-(4-imidazoleacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;
- 10 N-[1-(2(S),3-diaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;
- N-[1-(2(S),3-diaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;
- 15 N-[1-(3-aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;
- 20 N-[1-(3-aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(2(S)-amino-3-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

5 N-[1-(2(S)-amino-3-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

10 N-[1-(3-amino-2(S)-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

15 N-[1-(3-amino-2(S)-benzyloxycarbonylaminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(L-glutaminy)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

20 N-[1-(L-glutaminy)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(L-histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ;

25 N-[1-(L-histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(D-histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

30 N-[1-(D-histidyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(L-pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

5 N-[1-(L-pyroglutamyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester ;

2(S)-[1-(2(S)-pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

10 2(S)-[1-(2(S)-pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(2(S)-pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine isopropyl ester;

15 2(S)-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

20 2(S)-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(2(S)-pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine sulfone;

25 2(S)-[1-(2(S)-pyroglutamyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine sulfone methyl ester;

2(S)-[1-(pyrid-3-ylcarboxy)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

30 2(S)-[1-(pyrid-3-ylcarboxy)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

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2(R)-{2-[1-(naphth-2-yl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenylpropionyl-methionine;

5 2(R)-{2-[1-(naphth-2-yl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenylpropionyl-methionine methyl ester;

2(S)-[1-(pyrid-3-ylmethyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

10 2(S)-[1-(pyrid-3-ylmethyl)pyrrolidin-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

15 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone isopropyl ester;

20 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

25 N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ;

30 N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone methyl ester ;

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N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine sulfone;

5 N-[1-(sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

10 N-[1-(N,N-dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester ;

15 N-[1-(N,N-dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine methyl ester;

20 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

25 N-[1-(glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

30 N-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

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N-[1-(2-acetylamino-3(S)-benzyloxycarbonylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 5 N-[1-(2-acetylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(2-amino-3(S)-acetylamino-3(S)-aminopropionyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

10

2(S)-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine methyl ester;

15

2(S)-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy]-3-phenylpropionyl-methionine;

2(R)-{2-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester ;

20

2(R)-{2-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

2(R)-{2-[1-(4-nitrobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

25

2(R)-{2-[1-(4-nitrobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

30

2(R)-{2-[1-(4-methoxybenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

2(R)-{2-[1-(4-methoxybenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

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2(R)-{2-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy}-3-phenyl propionyl-methionine methyl ester;

5 2(R)-{2-[1-(4-cyanobenzyl)-1H-imidazol-5-ylacetyl]pyrrolidin-3(S)-ethyl-2(S)-ylmethoxy}-3-phenyl propionyl-methionine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetyl amino)alanine methyl ester;

10 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetyl amino)alanine;

N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetyl amino)alanine methyl ester;

15 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetyl amino)alanine;

20 N-[1-(seryl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(D-alanyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

25 N-[1-(1H-imidazol-4-carbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

30 N-[1-(isoasparagyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(1H-imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

35 N-[1-(3-pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(2-pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester ;

- 5 N-[1-(4-pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(seryl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

10

N-[1-(D-alanyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 15 N-[1-(1H-imidazol-4-carbonyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine ;

N-[1-(isoasparagyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 20 N-[1-(1H-imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(3-pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

25

N-[1-(2-pyridylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

- 30 N-[1-(4-pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylmethyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

- 35 N-[1-(2-aminoethyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine;

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N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(2-thienyl)alanine;

- 5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(trifluoromethyl)alanine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(2(S)-amino-4-acetylamino)butyric acid ;

10

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N,N-dimethyl)glutamine;

- 15 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine; -

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

- 20 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(4-methoxybenzyl)glycyl-methionine;

N-[1-(glycyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

- 25 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine;

N-((4-imidazolyl)methyl-(2S)-pyrrolidinylmethyl)-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

30

N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(2-thienyl)alanine methyl ester;

- 35 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N,N-dimethyl)glutamine methyl ester ;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(trifluoromethyl)alanine methyl ester;

- 5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(2(S)-amino-4-acetylamino)butyric acid methyl ester;

- 10 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine methyl ester;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine methyl ester;

- 15 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(4-methoxybenzyl)glycyl-methionine methyl ester;

- 20 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]- N-(benzyl)glycyl-methionine methyl ester;

N-[1-(glycyl) pyrrolidin-3(S)-ethyl-2(S)-ylmethyl]-N-(benzyl)glycyl-methionine methyl ester;

- 25 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine cyclohexyl ester;

- 30 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine benzyl ester;

- 35 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine ethyl ester;

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N-[1-(sarcosyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

5 N-[1-(N,N-dimethylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine (2-pyridylmethyl) ester;

10 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine (1-glyceryl) ester;

N-[1-L-prolylpyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

15 N-[1-(L-prolyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

20 N-[1-(1-morpholinoacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(1-morpholinoacetyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

25 N-[1-(4-piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(4-piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

30 N-[1-(3-piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

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N-[1-(3-piperidinecarbonyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

5 N-[1-(2-pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(2-pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

10 N-[1-(4-pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

N-[1-(4-pyridylglycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

15 N-[1-(4-pyridyl(N-methyl)glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine methyl ester;

20 N-[1-(4-pyridyl(N-methyl)glycyl)pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylpropionyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine;

25 N-[1-(1H-imidazol-4-ylpropionyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

N-[1-(4-pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine;

30 N-[1-(4-pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine methyl ester;

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N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -acetylamino)alanine cyclohexyl ester;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N-methyl)glutamine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-(N-methyl)glutamine methyl ester ;

10 N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylcarbonylamino)alanine;

15 N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylcarbonylamino)alanine methyl ester ;

N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylsulfonylamino)alanine;

20 N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -methylsulfonylamino)alanine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -propionylamino)alanine ;

25 N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -propionylamino)alanine methyl ester;

30 N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -pyrrolidinon-1-ylamino)alanine;

N-[1-(1H-imidazol-4-ylacetyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-(β -pyrrolidinon-1-ylamino)alanine methyl ester ;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

10 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine;

15 N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(3-methoxybenzyl)glycyl-methionine methyl ester;

20 N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

25 N-[1-(1H-imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methoxybenzyl)glycyl-methionine methyl ester;

30 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-cyanobenzyl)glycyl-methionine;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(3-cyanobenzyl)glycyl-methionine methyl ester ;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(4-cyanobenzyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

10 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

15 N-[1-(glycyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

20 N-[1-(1H-imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(2-cyanobenzyl)glycyl-methionine methyl ester;

25 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methylbenzyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-methylbenzyl)glycyl-methionine methyl ester;

30 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-trifluoromethylbenzyl)glycyl-methionine;

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N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(2-trifluoromethylbenzyl)glycyl-methionine methyl ester;

5 N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylsulfonyl)glycyl-methionine;

N-[1-(1H-imidazol-4-ylacetyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylsulfonyl)glycyl-methionine methyl ester;

10 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine 4-N-methylpiperidiny ester;

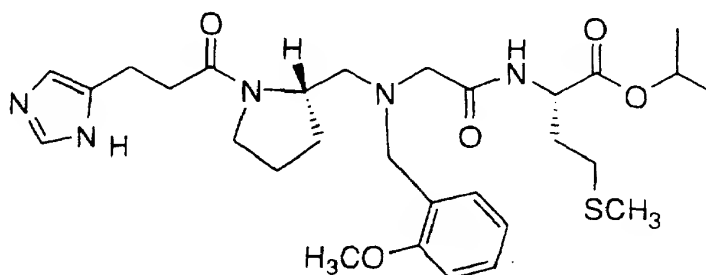
N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine tert-butyl ester;

15 N-[1-(glycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine 3-pentyl ester;

20 N-[1-(4-pyridylglycyl) pyrrolidin-2(S)-ylmethyl]-N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

N-[1-(1H-imidazol-4-ylpropionyl)pyrrolidin-2(S)-ylmethyl]- N-(1-naphthylmethyl)glycyl-methionine isopropyl ester;

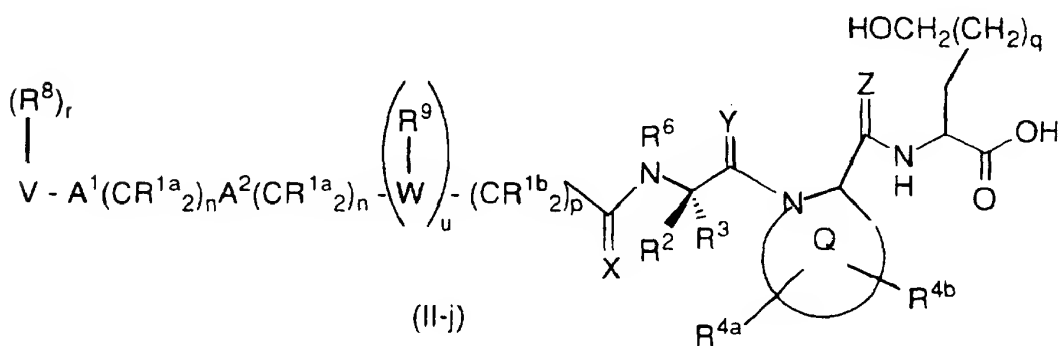
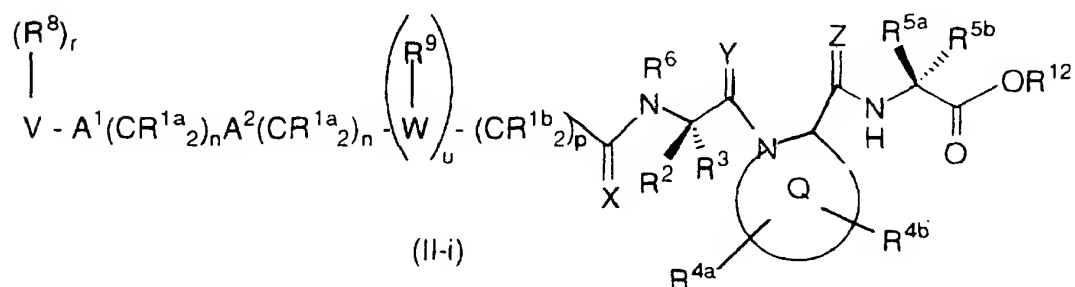
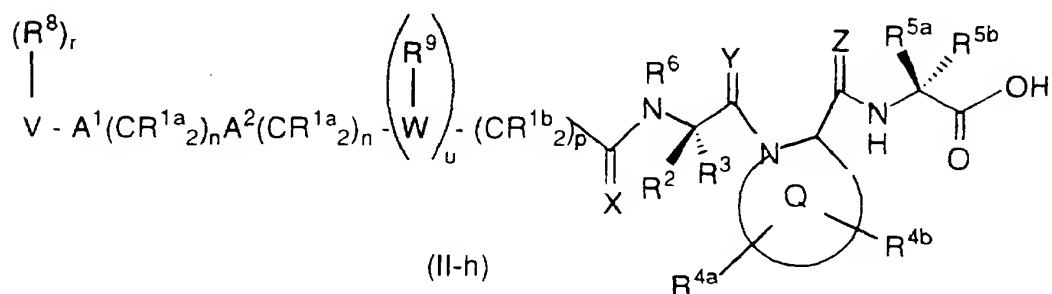
25 N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(2-methoxybenzyl)glycyl-methionine isopropyl ester



or a pharmaceutically acceptable salt thereof.

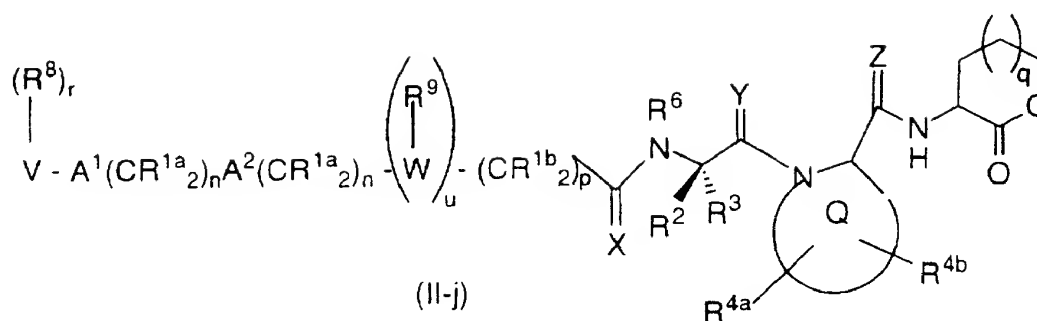
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11. A method of treating cancer in accordance with claim 6 wherein the farnesyl transferase inhibiting compound is
- 5 (c) a compound represented by one of formulas (II-h) through (II-k):



10

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selected from the group consisting of:

5

N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine methyl ester;

10

N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine;

15

N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-prolyl-methionine methyl ester;

N-[1-(1H-imidazol-4-ylacetyl)-3(S)-ethylpyrrolidin-2(S)-ylmethyl]-prolyl-methionine;

20

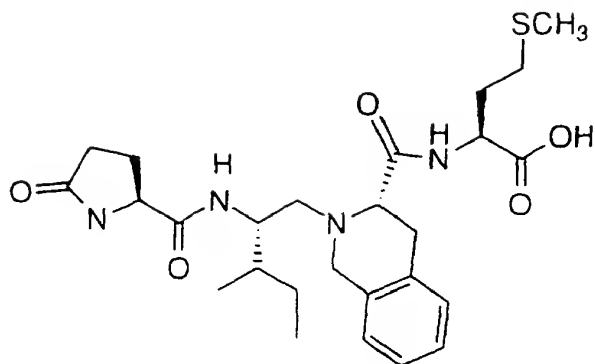
N-[1-glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine methyl ester;

N-[1-glycylpyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine;

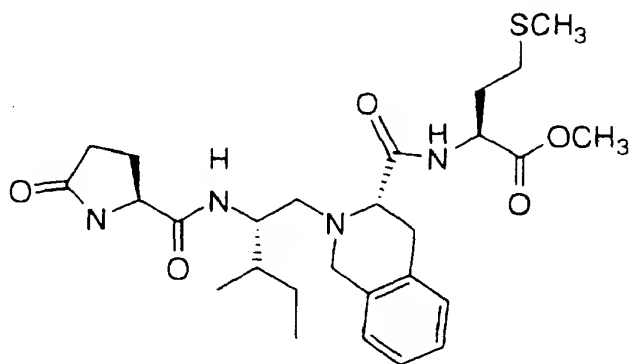
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N-[L-pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine

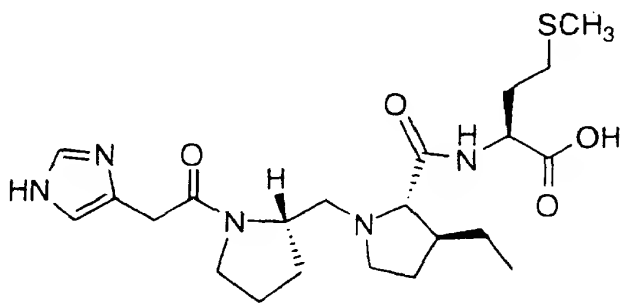
-308-



N-[L-pyroglutamyl-2(S)-amino-3(S)-methylpentyl]-1,2,3,4-tetrahydro-3(S)-isoquinolinecarbonyl-methionine methyl ester

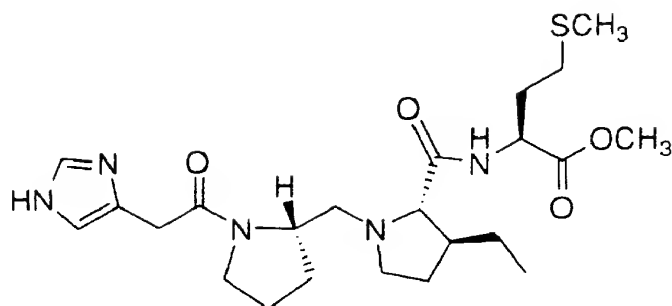


- 5 N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine

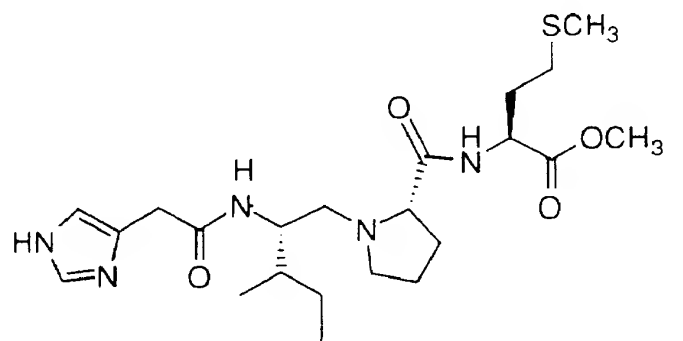


- N-[1-(1H-imidazol-4-ylacetyl)-pyrrolidin-2(S)-ylmethyl]-3(S)-ethylprolyl-methionine methyl ester

-309-

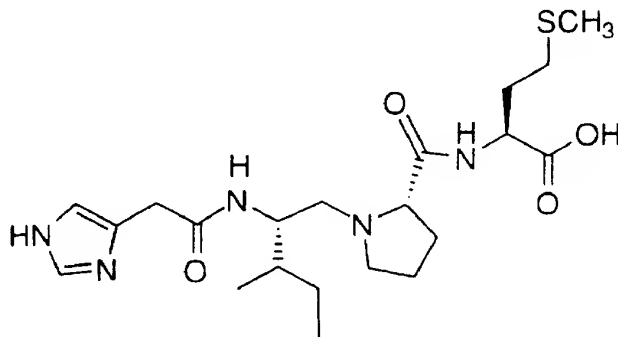


N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine methyl ester



5 and

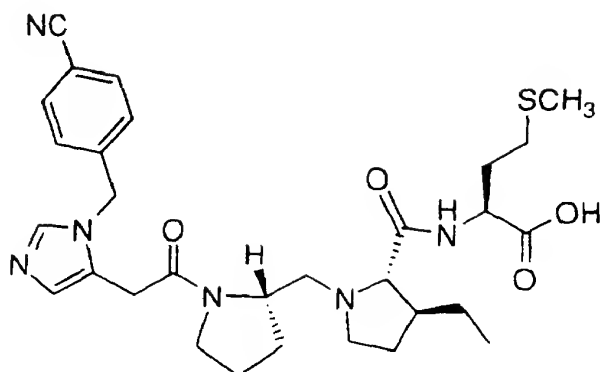
N-[(1H-imidazol-4-ylacetyl-2(S)-amino)-3(S)-methylpentyl]-prolyl-methionine



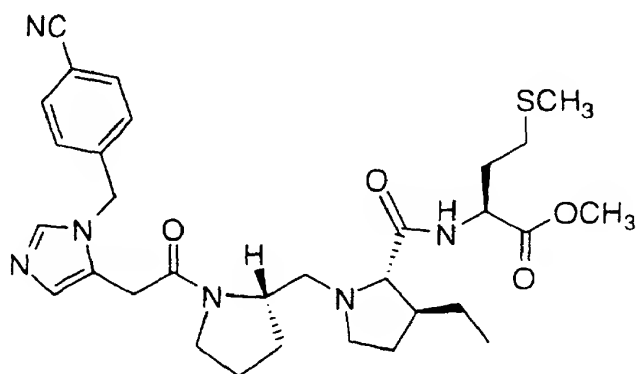
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(N-[1-cyanobenzyl]-1H-imidazol-5-yl)acetyl]pyrrolidin-2(S)-ylmethyl]-3(S)-ethyl-prolyl methionine;

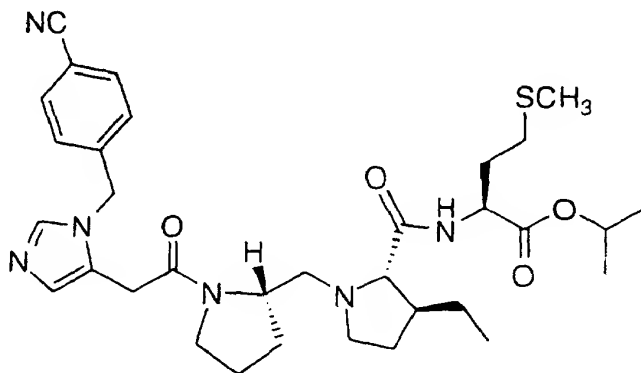
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(N-[1-cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-
 5 3(S)-ethyl-prolyl methionine methyl ester;



(N-[1-cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-
 10 3(S)-ethyl-prolyl methionine isopropyl ester, and



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or a pharmaceutically acceptable salt thereof.

12. A method in accordance with claim 1 wherein the farnesyl protein
5 transferase inhibiting compound is selected from the group consisting
of:

(S)-1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)-imidazolylmethyl]-5-[2-
(methanesulfonyl)ethyl]-2-piperazinone dihydrochloride;

10

1-(3-chlorophenyl)-4-[1-(4-cyanobenzyl)imidazolyl-methyl]-2-
piperazinone dihydrochloride;

- 15 N-[1-(1H-Imidazol-4-propionyl) pyrrolidin-2(S)-ylmethyl]-N-(2-
methoxybenzyl)glycyl-methionine isopropyl ester;

(N-[1-Cyanobenzyl)-1H-imidazol-5-yl]acetyl]pyrrolidin-2(S)-ylmethyl]-
3(S)-ethyl-prolyl methionine isopropyl ester;

- 20 2(S)-*n*-Butyl-1-[1-(4-cyanobenzyl)imidazol-5-ylmethyl]-4-(2,3-
dimethylphenyl)piperazin-5-one;

- N-[2(S)-N'-(1-(4-Cyanophenyl-methyl)-1H-imidazol-5-ylacetyl)amino-
3(S)-methylpentyl]-N-1-naphthylmethyl-glycyl-methionine methyl ester
25 and

- 2(S)-[2(S)-[2(R)-Amino-3-mercapto]propylamino-3(S)-methyl]-
pentyloxy-3-phenylpropionyl-methionine sulfone isopropyl ester,
30 or a pharmaceutically acceptable salt thereof.

| A. CLASSIFICATION OF SUBJECT MATTER IPC(6) A61K 31/44, 31/495 US CL 514/255, 341 According to International Patent Classification (IPC) or to both national classification and IPC | | |
|--|---|---|
| B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. 514/255, 341 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) | | |
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| (21) International Application Number: PCT/US97/07632 (22) International Filing Date: 8 May 1997 (08.05.97) (30) Priority Data: 08/644,544 10 May 1996 (10.05.96) US (71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US). (72) Inventors: ZHANG, Rumin; 4 Devon Road, Edison, NJ 08820 (US). MUI, Philip, W.; 1 Windswept Lane, Freehold, NJ 07728 (US). WEBER, Patricia, C.; 1970 Timber Lakes Drive, Yardley, PA 19067 (US). (74) Agents: DULAK, Norman, C. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US). | | (81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: SYNTHETIC INHIBITORS OF HEPATITIS C VIRUS NS3 PROTEASE (57) Abstract An inhibitor of the HCV NS3 protease. The inhibitor is a subsequence of a substrate of the NS3 protease or a subsequence of the NS4A cofactor. Another inhibitor of the present invention contains a subsequence of a substrate linked to a subsequences of the NS4A cofactor. In another embodiment the inhibitor is a bivalent inhibitor comprised of a subsequence, a mutated subsequence or a mutated full-length of a substrate of the NS3 protease linked to a subsequence, a mutated subsequence or a mutated full-length subsequence of the HCV NS4A cofactor. | | |

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SYNTHETIC INHIBITORS OF HEPATITIS C VIRUS NS3 PROTEASE

5

BACKGROUND OF THE INVENTION

10 Hepatitis C virus (HCV) is considered to be the major etiological agent of non-A non-B (NANB) hepatitis, chronic liver disease, and hepatocellular carcinoma (HCC) around the world. The viral infection accounts for greater than 90% of transfusion -associated hepatitis in U.S. and it is the predominant form of hepatitis in adults over 40 years of
15 age. Almost all of the infections result in chronic hepatitis and nearly 20% develop liver cirrhosis.

 The virus particle has not been identified due to the lack of an efficient *in vitro* replication system and the extremely low amount of
20 HCV particles in infected liver tissues or blood. However, molecular cloning of the viral genome has been accomplished by isolating the messenger RNA (mRNA) from the serum of infected chimpanzees then cloned using recombinant methodologies. [Grakoui A. *et al.* J. Virol. 67: 1385 - 1395 (1993)] It is now known that HCV contains a positive strand
25 RNA genome comprising approximately 9400 nucleotides, whose organization is similar to that of flaviviruses and pestiviruses. The genome of HCV, like that of flavi- and pestiviruses, encodes a single large polyprotein of about 3000 amino acids which undergoes proteolysis to form mature viral proteins in infected cells.

30

 Cell-free translation of the viral polyprotein and cell culture expression studies have established that the HCV polyprotein is processed by cellular and viral proteases to produce the putative structural and nonstructural (NS) proteins. At least nine mature viral
35 proteins are produced from the polyprotein by specific proteolysis. The order and nomenclature of the cleavage products are as follows: NH₂-C-E1-E2-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. The three amino terminal putative structural proteins, C (capsid), E1, and E2 (two

- 2 -

envelope glycoproteins), are believed to be cleaved by host signal peptidases of the endoplasmic reticulum(ER) . The host enzyme is also responsible for generating the amino terminus of NS2 . The proteolytic processing of the nonstructural proteins are carried out by the viral proteases: NS2-3 and NS3, contained within the viral polyprotein. The NS2-3 protease catalyzes the cleavage between NS2 and NS3. It is a metalloprotease and requires both NS2 and the protease domain of NS3. The NS3 protease catalyzes the rest of the cleavages of the substrates in the nonstructural part of the polyprotein. The NS3 protein contains 631 amino acid residues and is comprised of two enzymatic domains: the protease domain contained within amino acid residues 1-181 and a helicase ATPase domain contained within the rest of the protein. It is not known if the 70 kD NS3 protein is cleaved further in infected cells to separate the protease domain from the helicase domain, however, no cleavage has been observed in cell culture expression studies.

The NS3 protease is a member of the serine proteinase class of enzymes. It contains His, Asp, and Ser as the catalytic triad. Mutation of the catalytic triad residues abolishes the cleavages at substrates NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B. The cleavage between NS3 and NS4A is mediated through an intramolecular enzymatic reaction, whereas the cleavages at NS4A/4B, 4B/5A, 5A/5B sites occur in a *trans* enzymatic reaction.

Experiments using transient expression of various forms of HCV NS polyproteins in mammalian cells have established that the NS3 serine protease is necessary but not sufficient for efficient processing of all these cleavages. Like flaviviruses, the HCV NS3 protease also requires a cofactor to catalyze some of these cleavage reactions. In addition to the serine protease NS3, the NS4A protein is absolutely required for the cleavage of the substrate at the NS3/4A and 4B/5A sites and increases the efficiency of cleavage of the substrate between 5A/5B, and possibly 4A/4B.

Because the HCV NS3 protease cleaves the non-structural HCV proteins which are necessary for the HCV replication, the NS3 protease can be a target for the development of therapeutic agents against the

HCV virus. Thus there is a need for the development of inhibitors of the HCV protease.

SUMMARY OF THE INVENTION

5

The present invention fills this need by providing for a bivalent inhibitor of an hepatitis C NS3 protease comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide.

15 The present application further provides for an inhibitor of an HCV protease comprised of a peptide, said peptide being a subsequence, a mutated subsequence, or a mutated full-length sequence of a substrate of the HCV NS3 protease.

20 The present application further provides for an inhibitor of an HCV NS3 protease comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of an HCV NS4A polypeptide.

25 The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor being comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide.

35 The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor being comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor
5 being comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of an HCV NS4A polypeptide.

The present invention further comprises a pharmaceutical
10 composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being an inhibitor of an HCV NS3 protease, said inhibitor being comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, a mutated
15 subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide, and a pharmaceutical carrier.

The present invention further provides for a pharmaceutical
20 composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, said inhibitor being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

25
The present invention further provides for a pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, wherein said inhibitor
30 is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV NS4A polypeptide.

35

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically depicts an embodiment of a bivalent inhibitor of the present invention.

5

Figure 2 depicts the recombinant synthesis of plasmid pBJ1015.

Figure 3 depicts the recombinant synthesis of plasmid pTS56-9.

10 Figure 4 depicts the recombinant synthesis of plasmid pJB1006.

Figure 5 depicts the recombinant synthesis of plasmid pBJ1022.

15 Figure 6 depicts the recombinant synthesis of plasmid pNB(-V)182Δ4AHT.

Figure 7 depicts the recombinant synthesis of plasmid pT5His/HIV/183.

DETAILED DESCRIPTION OF THE INVENTION

20

The teachings of all references cited are incorporated herein in their entirety by reference.

25 The present invention are inhibitors of the HCV NS3 protease. The present invention relates to inhibitors of the HCV NS3 protease which inhibit either the interaction of a substrate or cofactor NS4A with the NS3 protease or a bivalent inhibitor which inhibits the interaction of the NS3 protease with both cofactor NS4A and a substrate of the NS3 protease. Compared to inhibitors targeting only at a single binding site,
30 bivalent enzyme inhibitors may provide additional advantages in terms of higher binding affinity (potency), as well as enhanced specificity against similar cellular host enzymes for reduced toxicity effects.

Design Strategy of Bivalent Inhibitors of HCV NS3 Protease

35

The basic strategy for the design of bivalent inhibitors of HCV NS3 protease involved the devise of a molecular framework consisting of three individual components:

1. a region appropriate for binding to a substrate binding site;
 2. a region suitable for binding to the NS4A binding site;
 3. a flexible linker region connecting regions (1) and (2) which
- 5 would allow the two end regions to bind to their respective binding sites.

Schematically, this is represented by Figure 1 in which the substrate subsequence is depicted as block, 10, being attached to linker 12, and said linker 12 being attached to the polypeptide NS4A designated 14.

Since the NS3 protease cleaves the HCV polyprotein at the NS3/4A, 4A/4B, 4B/5A and 5A/5B junctions, then subsequences of or mutated subsequences of these sites can be used as substrate inhibitors.

15 A substrate inhibitor which is a subsequence of the inhibitor should be a subsequence which is prior to or after the cleavage site but preferably should not contain the cleavage site. A mutated subsequence or mutated full-length sequence of the substrate can be used if the cleavage site is mutated so that the cleavage of the substrate does not occur cleavage

20 leads to mechanism-based inactivation of the protease.

For example, the NS3/4A cleavage site contains the following sequence:

25 Cys Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu
5 10 15
Val Gly Gly Val Leu (SEQ. ID NO.: 26)
20

The cleavage site is between the threonine at position 10 and the serine at position 11. Any subsequence inhibitor should preferably be before the serine or after the threonine residue. Alternatively, a mutated subsequence or sequence can be produced by changing the threonine/serine cleavage site at position 10-11 to eliminate the cleavage site.

- 7 -

NS4A/4B contains the following sequence.

```

Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
                    5                      10                      15
5   Tyr Ile Glu Gln Gly (SEQ ID NO.: 27).
                    20

```

The cleavage site is between the cysteine residue at position 10 and the serine at position 11. Any subsequence should preferably be before the serine or after the cysteine, but should preferably not contain both the cysteine and the serine. Alternatively, a mutated subsequence or sequence can be produced by changing the cysteine/serine cleavage site at position 10 - 11 to eliminate the cleavage site.

15 NS4B/5A contains the following sequence.

```

Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp Leu
                    5                      10                      15
20  Arg Asp Ile Trp Asp (SEQ ID NO.: 28)
                    20

```

The cleavage site is between the cysteine at position 10 and serine at position 11. Any subsequence should preferably end before the serine or start after the cysteine but should preferably not contain both the serine and the cysteine. Alternatively, a mutated subsequence or sequence can be produced by changing the cysteine/serine cleavage sit at position 10 - 11 to eliminate the cleavage site.

NS5A/5B contains the following sequence.

```

30  Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr
                    5                      10                      15
    Gly (SEQ. ID NO.: 25)

```

35 The cleavage site is between the cysteine at position 8 and the serine at position 9. Any subsequence should preferably end at the cysteine or start at the serine, but should preferably not contain both the cysteine and the serine. Alternatively, a mutated sequence or subsequence can be

- 8 -

produced by changing the cysteine/serine cleavage site at position 8 - 9 to eliminate the cleavage site.

5 Linker 12 can be any chemical entity that can form a bond with polypeptides 10 and 14. Preferably the linker should be equivalent in length to a carbon chain having about 7-14 carbon residues. Examples of suitable linkers are two 6-aminocaproic acid (Acp) residues or an Acp and Lys wherein one of the polypeptides 10 or 14 form a peptide bond
10 with the ϵ amine of lysine.

Examples of bivalent inhibitors of the present invention are the following:

15 Glu-Asp-Val-Val-Cys-Cys-Acp-Acp-Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 1)

 Glu-Asp-Val-Val-Cys-Cys-Acp-Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys-Lys (SEQ ID NO:2)

20 Glu-Asp-Val-Val-Cys-Cys-Acp-Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 3)

 wherein Xaa is a lysine residue having a peptide bond between its
25 ϵ -amino and the carboxyl group of the following lysine which forms a peptide bond with the glycine at position 10. Furthermore, the glutamic acid residue at position 1 may or may not be acetylated.

30 Glu-Asp-Val-Val-Cys-Cys-Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 4)

 wherein Xaa is Lysine having a peptide bond between its ϵ -amino and the carboxyl group of the following lysine which forms a peptide bond with the Gly; furthermore, the carboxyl group of the Xaa forms a
35 peptide bond with the α -amino group of another lysine (not shown);

 Glu-Asp-Val-Val-Cys-Cys-Acp-Acp-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 5)

- 9 -

wherein the amino acids at positions 9-21 are preferably D-amino acids;

5 Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-Cys-Val-Val-Ile-
Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 6)

wherein the lysine residue at position 8 has a peptide bond between the carboxyl of Acp and the α amino group of the lysine, and
10 the ϵ amino group of the lysine at position 8 forms a peptide bond with the carboxyl group of the cysteine residue at position 9 and the amino acid residues at positions 9-21 are preferably D-amino acid residues;

Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-Gly-Ser-Leu-Val-Ile-Arg-
15 Gly-Val-Ile-Val-Val-Cys-Lys (SEQ ID NO: 7)

wherein amino acid residues at positions 8-20 are preferably D-amino acid residues;

20 Glu-Asp-Val-Val-Cys-Cys-Xaa-Cys-Val-Val-Ile-Val-Gly-
Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 8)

wherein Xaa is a Lys which forms a peptide bond between its ϵ -amino acid and the carboxyl group of the Cys residue at position 8 and
25 the carboxyl group of the Lys residue forms a peptide bond with an alpha amino group of another Lys residue (not shown), preferably the amino acid residues at positions 8 - 20 are D- amino acids.

Examples of suitable monovalent inhibitors of the present
30 invention are the following:

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys-Lys
(SEQ ID NO.: 9)

wherein the amino acid residues at positions 1- 13 are preferably
35 D-amino acid residues;

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Lys (SEQ ID NO.: 10)

wherein amino acid residues at positions 1 - 11 are preferably D-amino acid residues;

Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys
(SEQ ID NO.: 11)

5 wherein the amino acid residues are preferably D-amino acid residues;

Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val (SEQ ID NO.: 12)

10 wherein the amino acid residues are preferably D-amino acid residues and the serine residue at position 1 has been preferably acetylated;

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys
(SEQ ID NO.: 13)

15 wherein the amino acid residues are preferably D-amino acid residues the lysine residue at position 1 is preferably acetylated;

Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile Val-Val-Cys-Lys-Lys
(SEQ ID NO.: 14);

20 wherein Xaa is biotin and the amino acid residues at positions 2 - 14 are preferably D-amino acid residues;

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys-Xaa-Lys
(SEQ ID NO.: 15);

25

Xaa is a lysine residue in which the ϵ amino group of the lysine forms a peptide bond with a biotin, and amino acid residues at positions 1 - 13 are preferably D-amino acid residues.

30

The inhibitors of the present invention can be synthesized by a suitable method such as by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis.

35 The polypeptides are preferably prepared by solid phase peptide synthesis as described by Merrifield, J. Am. Chem. Soc. 85:2149 (1963). The synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-

- 11 -

chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the polypeptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The
5 conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise polypeptide synthesis. Included are
10 acyl type protecting groups (*e.g.*, formyl, trifluoroacetyl, acetyl), aryl type protecting groups (*e.g.*, biotinyl), aromatic urethane type protecting groups [*e.g.*, benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxy-carbonyl (Fmoc)],
15 aliphatic urethane protecting groups [*e.g.*, t-butyloxycarbonyl (tBoc), isopropylloxycarbonyl, cyclohexyloxycarbonyl] and alkyl type protecting groups (*e.g.*, benzyl, triphenylmethyl). The preferred protecting groups are tBoc and Fmoc, thus the peptides are said to be synthesized by tBoc and Fmoc chemistry, respectively.

20 The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction
25 conditions that will not alter the finished polypeptide. In tBoc chemistry, the side-chain protecting groups for trifunctional amino acids are mostly benzyl based. In Fmoc chemistry, they are mostly tert.-butyl or trityl based.

30 In tBoc chemistry, the preferred side-chain protecting groups are tosyl for Arg, cyclohexyl for Asp, 4-methylbenzyl (and acetamidomethyl) for Cys, benzyl for Glu, Ser and Thr, benzyloxymethyl (and dinitrophenyl) for His, 2-Cl-benzyloxycarbonyl for Lys, formyl for Trp and 2-bromobenzyl for Tyr. In Fmoc
35 chemistry, the preferred side-chain protecting groups are 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg, trityl for

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Asn, Cys, Gln and His, tert-butyl for Asp, Glu, Ser, Thr and Tyr, tBoc for Lys and Trp.

- 5 Solid phase synthesis is usually carried out from the carboxyl-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl, chlorotriyl or hydroxymethyl resin, and the resulting polypeptide will have a
- 10 free carboxyl group at the C-terminus. Alternatively, when an amide resin such as benzhydrylamine or p-methylbenzhydrylamine resin (for tBoc chemistry) and Rink amide or PAL resin (for Fmoc chemistry) is used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These
- 15 resins, whether polystyrene- or polyamide-based or polyethyleneglycol-grafted, with or without a handle or linker, with or without the first amino acid attached, are commercially available, and their preparations have been described by Stewart et al (1984)., "Solid Phase Peptide Synthesis" (2nd Edition), Pierce Chemical Co.,
- 20 Rockford, IL.; and Bayer & Rapp (1986) *Chem. Pept. Prot.* 3, 3; and Atherton, et al. (1989) *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford.

- The C-terminal amino acid, protected at the side-chain if
- 25 necessary and at the alpha-amino group, is attached to a hydroxymethyl resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIPCDI) and carbonyldiimidazole (CDI). It can be attached to chloromethyl or chlorotriyl resin directly in its cesium
- 30 tetramethylammonium salt form or in the presence of triethylamine (TEA) or diisopropylethylamine (DIEA). First amino acid attachment to an amide resin is the same as amide bond formation during coupling reactions

- 35 Following the attachment to the resin support, the alpha-amino protecting group is removed using various reagents depending on the protecting chemistry (e.g. , tBoc, Fmoc). The extent of Fmoc removal can be monitored at 300-320 nm or by a

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conductivity cell. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

5 Various activating agents can be used for the coupling reactions including DCC, DIPCDI, 2-chloro-1,3-dimethylimidium hexafluorophosphate (CIP), benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and its pyrrolidine analog (PyBOP), bromo-tris-pyrrolidino-phosphonium
10 hexafluorophosphate (PyBroP), N- [(1H-benzotriazol-1-yl) - (dimethylamino) methylene] -N-methylmethanaminium hexafluorophosphate N-oxide (HBTU) and its tetrafluoroborate analog (TBTU) or its pyrrolidine analog (HBPYU), (HATU) and its tetrafluoroborate analog (TATU) or pyrrolidine analog (HAPYU). The
15 most common catalytic additives used in coupling reactions include 4-dimethylaminopyridine (DMAP), 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HODhbt), N-hydroxybenzotriazole (HOBT) and 1-hydroxy-7-azabenzotriazole (HOAt). Amino acid fluorides or chlorides may be used for difficult couplings. Each protected amino
20 acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling reaction can be monitored at each stage, *e.g.*, by the ninhydrin reaction as described by Kaiser *et al.*, *Anal. Biochem.* 34:595 (1970). In cases
25 where incomplete coupling is found, the coupling reaction is extended and repeated and may have chaotropic salts added. The coupling reactions can be performed automatically with commercially available instruments such as ABI model 430A, 431A and 433A peptide synthesizers.

30

 After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent with proper scavengers. The Fmoc peptides are usually cleaved and deprotected by TFA with scavengers (*e.g.*, H₂O, ethanedithiol, phenol and thioanisole). The tBoc peptides
35 are usually cleaved and deprotected with liquid HF for 1-2 hours at -5 to 0°C, which cleaves the polypeptide from the resin and removes most of the side-chain protecting groups. Scavengers such as anisole, dimethylsulfide and p-thiocresol are usually used with the liquid HF

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to prevent cations formed during the cleavage from alkylating and acylating the amino acid residues present in the polypeptide. The formyl group of Trp and dinitrophenyl group of His need to be removed, respectively, by piperidine and thiophenol in DMF prior to the HF cleavage. The acetamidomethyl group of Cys can be removed by mercury(II) acetate and alternatively by iodine, thallium (III) trifluoroacetate or silver tetrafluoroborate which simultaneously oxidize cysteine to cystine. Other strong acids used for tBoc peptide cleavage and deprotection include trifluoromethanesulfonic acid (TFMSA) and trimethylsilyltrifluoroacetate (TMSOTf).

In particular the peptides of the present invention were assembled from a Fmoc-Amide resin or a Fmoc-L-Lys- (tBoc) - Wang resin on an ABI model 433A synthesizer (Applied Biosystems, Foster City, CA) by solid phase peptide synthesis method as originally described by Merrifield, J. Am.Chem.Soc. 85:2149 (1963) but with Fmoc chemistry. The side chains of trifunctional amino acids were protected by tert.-butyl for Glu, Asp and Ser, trityl for Cys, tert.-butyloxycarbonyl (tBoc) for Lys and 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg. N-a-Fmoc protected amino acids were pre-activated by HATU and 1-hydroxy-7-azabenzotriazole (HOAt) prior to coupling to the resin. Dimethylsulfoxide (20%) was added during conditional extended coupling and Fmoc deprotection reactions. The synthesis of the inhibitors SEQ ID NOs: 1, 2, 5, 7, and 9-15 was accomplished by sequential and linear assembly of appropriate D- and L-amino acids and achiral amino acids (Gly and Ahx). The synthesis of the inhibitors SEQ ID NOs: 3, 4, 6, and 8 required orthogonal chain assembly anchored at a Lys residue whose side chain amino group was protected by 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-ethyl (Dde). For example, for the preparation of the inhibitor SEQ ID NO: 3, Ac-Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-(Amide resin) (SEQ ID NO: 29) was first assembled. Then the Dde protecting group on the Lys residue was removed by 2% hydrazine in dimethylformamide (Bycroft, B.W. et al J. Chem. Soc. Chem. Commun. 1993, 778). Finally the second arm Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO:30) was sequentially assembled from the side chain amino group. The assembled peptide was cleaved from the resin with simultaneous

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deprotection of side chain protecting groups for three hours by trifluoroacetic acid (TFA) with proper scavengers (80% TFA : 4% phenol : 4% H₂O, 4% thioanisole : 4% ethanedithiol : 4% triisopropylsilane).

5 The cleaved peptide was separated from the resin by filtration and precipitated and repeatedly washed in anhydrous ethyl ether. The precipitated peptide was lyophilized in H₂O overnight. The lyophilized crude peptide was purified by reverse phase HPLC. The purified peptide was further analyzed by HPLC, mass spectroscopy and amino acid analysis.

10

One can ascertain if a potential compound is effective as an inhibitor of the HCV NS3 protease by using a high throughput assay utilizing the NS3 protease, the NS4 cofactor and the peptide substrates, either 4B/5A or 5A/5B. These can be used to screen for compounds
15 which inhibit proteolytic activity of the protease. One does this by developing techniques for determining whether or not a compound will inhibit the NS3 protease from cleaving the viral substrates. If the substrates are not cleaved, the virus cannot replicate. One example of such a high throughput assay is the scintillation proximity assay (SPA).
20 SPA technology involves the use of beads coated with scintillant. Bound to the beads are acceptor molecules such as antibodies, receptors or enzyme substrates which interact with ligands or enzymes in a reversible manner.

25 For a typical SPA based protease assay the substrate peptide is biotinylated at one end and the other end is radiolabelled with low energy emitters such as ¹²⁵I or ³H. The labeled substrate is then incubated with the enzyme. Avidin coated SPA beads are then added which bind to the biotin. When the substrate peptide is cleaved by the
30 protease, the radioactive emitter is no longer in proximity to the scintillant bead and no light emission takes place. Inhibitors of the protease will leave the substrate intact and can be identified by the resulting light emission which takes place in their presence.

35 Another example of a suitable assay technique is an HPLC assay in which the resultant reaction mixture containing the NS3 protease, the substrate products and the potential inhibitor is resolved on an HPLC column to determine the extent of the cleavage of the substrate. If the

substrate has not been cleaved or the cleavage has been inhibited, then only the intact substrate would be present or a reduced amount of the cleaved product will be shown to be present. If this is the case, then the compound is an effective inhibitor of the NS3 protease.

5

Pharmaceutical Compositions

The dosage level of inhibitors necessary for effective therapy to inhibit the HCV NS3 protease will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents.

Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. See also Langer (1990) Science 249:1527-1533.

Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. 1 μ g per kilogram weight of the patient to 500 mg per kilogram weight of the patient with an appropriate carrier is a range from which the dosage can be chosen. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

The inhibitors of the HCV NS3 protease of the present invention may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is

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preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press, Parrytown, NY; Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications 2d ed., Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets 2d ed., Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

The following examples are included to illustrate but not to limit the present invention.

Example 1

Bivalent Inhibitors of HCV NS3 Protease

The bivalent inhibitors of defined by SEQ ID NOs.: 1-10 were synthetically produced as described above and tested for their ability to inhibit the HCV NS3 protease as follows.

Into an aqueous solution containing 25 mM TRIS, 50 mM NaCl, .5 mM EDTA, 10% glycerol and .1% NP40 was placed the potential inhibitor, the HCV NS3 protease at a concentration of 0.05 μ M - 0.1 mM, the HCV NS4A cofactor at a concentration of 0.05 μ M - 0.1 μ M and the 5A/5B substrate at a concentration of 50 μ M. This solution was then incubated for approximately 2 hours at 30°C after which the solution was applied to an HPLC to determine if the 5A/5B remained intact and

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thus the compound was determined to be an inhibitor. However, if the HPLC showed that 5A and 5B were present without the 5A/5B then the compound is not an inhibitor. The potential inhibitors were assayed at several different concentrations to determine the concentration which produced 50% inhibition of the HCV NS3 protease. The results are shown below.

| | <u>Inhibitor</u> | <u>IC₅₀ (μM)</u> |
|----|------------------|-----------------------------|
| | SEQ ID NO:1 | 0.6 |
| | 50571-120 | |
| 10 | SEQ ID NO:2 | 3.0 |
| | 50962-13 | |
| | SEQ ID NO:3 | 3.0 |
| | 50828-001 | |
| | SEQ ID NO:4 | 3 - 30 |
| 15 | 50962-22 | |
| | SEQ ID NO:5 | 0.2 |
| | 50571-144 | |
| | SEQ ID NO:6 | 2.0 |
| | 50571-150 | |
| 20 | SEQ ID NO:7 | 0.2 |
| | 50828-131 | |
| | SEQ ID NO:8 | 0.2 |
| | 50962-24 | |

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Example 2**Monovalent Inhibitors of the HCV NS3 Protease**

5 Examples of monovalent inhibitors of the HCV NS3 protease are as follows.

| | Inhibitor | IC ₅₀ (μ M) |
|----|----------------|-----------------------------|
| 10 | SEQ ID NO.: 9 | 0.2 |
| | 50828-129 | |
| | SEQ ID NO.: 10 | 5 |
| | 50962-004 | |
| | SEQ ID NO.: 11 | 0.2 |
| 15 | 50828-70 | |
| | SEQ ID NO.: 12 | 0.6 |
| | 50828-116 | |
| | SEQ ID NO.: 13 | 2.0 |
| | 50571-147 | |
| 20 | SEQ ID NO.: 14 | 0.4 |
| | 50962-047 | |
| | SEQ ID NO.: 15 | 0.4 |
| | 50962-050 | |

25

Examples 3

30

Production of HCV NS3 Protease**A. Plasmid constructions.**

35 Several plasmids were designed and constructed using standard recombinant DNA techniques (Sambrook, Fritsch & Maniatis) to express the HCV protease in *E. coli* (Fig 2-7). All HCV specific sequences originated from the parental plasmid pBRTM/HCV 1-3011 (Grakoui *et*

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al.1993). To express the N-terminal 183 amino acid versions of the protease, a stop codon was inserted into the HCV genome using synthetic oligonucleotides (Fig. 3). The plasmids designed to express the N-terminal 246 amino acid residues were generated by the natural NcoI
5 restriction site at the C-terminus.

i) Construction of the plasmid pBJ1015 (Figure 2)

The plasmid pBRTM/HCV 1-3011 containing the entire HCV genome
10 (Grakoui A., *et al.*, *J. Virol.* 67: 1385-1395) was digested with the restriction enzymes Sca I and Hpa I and the 7138 bp (base pair) DNA fragment was isolated and cloned to the Sma I site of pSP72 (Promega) to produce the plasmid, pRJ201. The plasmid pRJ 201 was digested with Msc I and the 2106 bp Msc I fragment was isolated and cloned into the
15 Sma I site of the plasmid pBD7. The resulting plasmid pMBM48 was digested with Kas I and Nco I, and the 734 bp DNA fragment after blunt ending with Klenow polymerase was isolated and cloned into Nco I digested, klenow polymerase treated pTrc HIS B seq expression plasmid (Invitrogen). The ligation regenerated a Nco I site at the 5' end and Nsi I
20 site at the 3' end of HCV sequence. The plasmid pTHB HCV NS3 was then digested with Nco I and Nsi I, and treated with klenow polymerase and T4 DNA polymerase, to produce a blunt ended 738 bp DNA fragment which was isolated and cloned into Asp I cut, klenow polymerase treated expression plasmid pQE30 (HIV). The resulting
25 plasmid pBJ 1015 expresses HCV NS3 (246 amino acids) protease.

(ii) Construction of the plasmid pTS 56-9 with a stop codon after amino acid 183 (Figure 3)

30 The plasmid pTHB HCV NS3 was digested with Nco I, treated with klenow polymerase, then digested with Bst Y I; and the DNA fragment containing HCV sequence was isolated and cloned into Sma I and Bgl II digested pSP72. The resulting plasmid pTS 49-27 was then digested with Bgl II and Hpa I and ligated with a double stranded
35 oligonucleotide:

GA TCA CCG GTC TAG ATCT

T GGC CAG ATC TAGA (SEQ ID NO 18) to produce pTS 56-9.

Thus, a stop codon was placed directly at the end of DNA encoding the

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protease catalytic domain of the NS3 protein. This enabled the HCV protease to be expressed independently from the helicase domain of the NS3 protein.

- 5 (iii) Construction of the plasmid pJB 1006 Fused with a peptide of positively charged amino acids at the carboxy terminus of NS3 183 (Figure 4).

10 The plasmid pTS 56-9 was digested with Sph I and Bgl II and the DNA fragment containing HCV sequence was isolated and cloned into a Sph I, Bgl II cut pSP72. The resulting plasmid pJB 1002 digested with Age I and HpaI and ligated to a double stranded oligonucleotide,

```

      CCG  GTC  CGG  AAG  AAA  AAG  AGA  CGC  TAG  C
      AG  GCC  TTC  TTT  TTC  TCT  GCG  ATC  G

```

- 15 (SEQ ID NO 19), to construct pJB 1006. This fused the hydrophilic, solubilizing motif onto the NS3 protease.

- 20 (iv) Construction of the plasmid pBJ 1022 expressing His-NS3(183)-HT in E.coli (Figure 5)

25 The plasmid pJB 1006 was digested with NgoM I and Nhe I and the 216 bp DNA fragment was isolated and cloned into Ngo M I, Nhe I cut pBJ 1015 to construct plasmid pBJ 1019. The plasmid pBJ 1019 was digested with Nar I and Pvu II, and treated with Klenow polymerase to fill in 5' ends of Nar I fragments. The expression plasmid pQE31 (Invitrogen) was digested with BamH I, blunt ended with Klenow polymerase. The 717 bp Nar I- Pvu II DNA fragment was isolated and ligated to the 2787 bp BamH I/Klenowed -Msc I (Bal I) fragment of the expression plasmid pQE31 (Invitrogen). The recombinant plasmid, pBJ 1022, obtained after transformation into *E.coli* expresses His NS3(2-183)-HT which does not contain any HIV protease cleavage site sequence. The plasmid also contains a large deletion in the CAT (Chloramphenicol Acetyl Transferase) gene.

35

- (v) Construction of the plasmid pNB(-V)182-Δ4A HT (Figure 6)

- 22 -

The plasmid pMBM 48 was digested with Eag I and Xho I, treated with Klenow polymerase and the 320 bp DNA fragment was isolated and cloned into BamH I cut, blunt ended pSP 72 to construct the plasmid pJB1004. The 320 bp fragment encodes 7 amino acid from carboxy terminal of NS3(631), all of NS4A, and the amino terminal 46 amino acid of NS4B. The recombinant plasmid pJB1004 was digested with Eag I and Cel 2, blunt ended with Klenow polymerase. The 220 bp DNA fragment was isolated and cloned into the expression plasmid pQE30 which was digested with BamH I and blunt ended with Klenow polymerase prior to ligation. The resulting plasmid pJB 1011 was digested with NgoM I and Hind III and ligated to a double stranded oligonucleotide ,

15 CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAA TTC
GT TAA TAT GGA CTG TCC CTC CAA GAG ATG GTC CTT AAG

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC A
CTA CTC TAC CTT CTC ACG GCC TTC TTT TTC TCT GCG TTC GA
(SEQ ID NO 20)

20 to construct the plasmid pNB 4A HT. The plasmid pNB 4AHT was digested with Msl I and Xba I. The 1218 bp DNA fragment was isolated and cloned into Age I cut, klenow polymerase treated, Xba I cut vector DNA of pBJ 1019. The ligation results in a substitution of the 183rd amino acid residue valine by a glycine residue in NS3, and a deletion of amino terminal three amino acid residues of NS4A at the junction. The recombinant plasmid pNB182Δ4A HT comprising NS3(182aa)-G-NS4A(4-54 amino acid) does not contain NS3/NS4A cleavage site sequence at the junction and is not cleaved by the autocatalytic activity of NS3. Finally the plasmid pNB182Δ4A HT (SEQ ID NO 8) was digested with Stu I and Nhe I, the 803 bp DNA fragment was isolated and cloned into Stu I and Nhe I cut plasmid pBJ 1022. The resulting plasmid pNB(-V)182-Δ4A HT contains a deletion of the HIV sequence from the amino terminus end of the NS3 sequence and in the CAT gene (SEQ ID NO 23).

35

(vi) Construction of the plasmid pT5 His HIV-NS3 (Figure 7)

- 23 -

The plasmid pTS56-9 was digested with Bgl II, and treated with Klenow polymerase to fill in 5' ends. The plasmid was then digested with NgoM I and the blunt ended Bgl II/NgoMI fragment containing the NS3 sequence was isolated and ligated to the SglI, Klenow treated
5 NgmMI cut and Sal I klenowed pBJ 1015. The resulting plasmid is designated pT5His HIV 183.

Example 4

10

Purification of HCV NS3 Protease having a Solubilizing Motif

Purification of His182HT (SEQ ID NO 4) and

His (-V)182Δ4AHT (SEQ ID NO 8)

15

The recombinant plasmids pBJ1022 and pNB(-V)182Δ4A were used to transform separate cultures of *E. coli* strain M15 [pREP4] (Qiagen), which over-expresses the *lac* repressor, according to methods recommended by the manufacturer. M15 [pREP4] bacteria harboring
20 recombinant plasmids were grown overnight in broth containing 20g/L bactotrypton, 10g/L bacto-yeast extract, 5g/L NaCl (20-10-5 broth) and supplemented with 100μg/ml ampicillin and 25μg/ml kanamycin. Cultures were diluted down to O.D.600 of 0.1, then grown at 30°C to O.D.600 of 0.6 to 0.8, after which IPTG was added to a final concentration
25 of 1mM. At post-induction 2 to 3 hours, the cells were harvested by pelleting, and the cell pellets were washed with 100mM Tris, pH 7.5. Cell lysates were prepared as follows: to each ml equivalent of pelleted fermentation broth was added 50μl sonication buffer (50mM sodium phosphate, pH 7.8, 0.3M NaCl) with 1mg/ml lysozyme; cell suspension
30 was placed on ice for 30 min. Suspension was then brought to a final concentration of 0.2% Tween-20, 10mM dithiothreitol (DTT), and sonicated until cell breakage was complete. Insoluble material was pelleted at 12,000 x g in a microcentrifuge for 15 minutes, the soluble portion was removed to a separate tube and the soluble lysate was then
35 brought to a final concentration of 10% glycerol. Soluble lysates from cells expressing the plasmids produce strongly immunoreactive bands of the predicted molecular weight. Soluble lysates prepared for Ni²⁺

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column purification were prepared with 10mM β -mercaptoethanol (BME) instead of DTT. Lysates were stored at -80°C .

5 Purification using Ni^{2+} -Nitrosyl acetic acid (NTA) agarose (OIA GEN)

The proteins were then purified by placing the extracted lysate on an NTA agarose column. NTA agarose column chromatography was used because the histidine tag which was fused to the N-terminus of the proteases readily binds to the nickel column. This produces a powerful affinity chromatographic technique for rapidly purifying the soluble protease. The column chromatography was performed in a batch mode. The Ni^{2+} NTA resin (3ml) was washed twice with 50 ml of Buffer A (50mM sodium phosphate pH 7.8 containing 10% glycerol, 0.2% Tween-20, 10mM BME). The lysate obtained from a 250 ml fermentation (12.5 ml) was incubated with the resin for one hour at 4°C . The flow through was collected by centrifugation. The resin was packed into a 1.0 x 4 cm column and washed with buffer A until the baseline was reached. The bound protein was then eluted with a 20 ml gradient of imidazole (0-0.5M) in buffer A. Eluted fractions were evaluated by SDS-PAGE and western blot analysis using a rabbit polyclonal antibody to His-HIV 183.

Purification using POROS metal-chelate affinity column

25 In an alternative method to purify the proteins the lysate containing the proteins were applied to a POROS metal-chelate affinity column. Perfusion chromatography was performed on a POROS MC metal chelate column (4.6 x 50mm, 1.7 ml) precharged with Ni^{2+} . The sample was applied at 10 ml/min and the column was washed with buffer A. 30 The column was step eluted with ten column volumes of buffer A containing 25 mM imidazole. The column was further eluted with a 25 column volume gradient of 25-250 mM imidazole in buffer A. All eluted fractions were evaluated by SDS-PAGE and western blot analysis using rabbit polyclonal antibody.

35

Example 5

Peptide Synthesis of the 5A/5B and 4B/5A Substrates

- 25 -

The peptides 5A/5B and 4B/5A substrates (SEQ ID NOs 16, 18, 19, 20 and 21) were synthesized using Fmoc chemistry on an ABI model 431A peptide synthesizer. The manufacture recommended FastMoc™ activation strategy (HBTU/HOBt) was used for the synthesis of 4A activator peptide. A more powerful activator, HATU with or without the additive HOAt were employed to assemble 5A/5B substrate peptides on a preloaded Wang resin. The peptides were cleaved off the resin and deprotected by standard TFA cleavage protocol. The peptides were purified on reverse phase HPLC and confirmed by mass spectrometric analysis.

Example 6

HPLC-assay using a synthetic 5A/5B peptide substrate

To test the proteolytic activity of the HCV NS3 protease the DTEDVVCC SMSYTWGK (SEQ ID NO 16) and soluble HCV NS3 (SEQ ID NO 27) were placed together in an assay buffer. The assay buffer was 50mM sodium phosphate pH 7.8, containing 15% glycerol, 10mM DTT, 0.2% Tween20 and 200 mM NaCl). The protease activity of SEQ ID NO 27 cleaved the substrate into two byproduct peptides, namely 5A and 5B. The substrate and two byproduct peptides were separated on a reversed-phase HPLC column. (Dynamax, 4.6 x 250 mm) with a pore size of 300Å and a particle size of 5µm. The column was equilibrated with 0.1%TFA (Solvent A) at a flow rate of 1 ml per minute. The substrate and the product peptide standards were applied to the column equilibrated in A. Elution was performed with a acetonitrile gradient (Solvent B=100% acetonitrile in A). Two gradients were used for elution (5% to 70%B in 50 minutes followed by 70% to 100%B in 10 minutes).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: Schering Corp.

(ii) TITLE OF INVENTION: Synthetic Inhibitors of Hepatitis C Virus
NS3 Protease

10 (iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering Corp.
(B) STREET: 2000 Galloping Hill Road
15 (C) CITY: Kenilworth
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07033-0530

20 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 7.1
(D) SOFTWARE: Microsoft Word 5.1a

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

30

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/644,544
(B) FILING DATE: 10 May 1996

35 (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Dulak, Norman C.
(B) REGISTRATION NUMBER: 31,608

- 27 -

(C) REFERENCE/DOCKET NUMBER: JB0595

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 908-298-5061

5 (B) TELEFAX: 908-298-5388

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

20 Glu Asp Val Val Cys Cys Acp Acp Cys Val Val Ile Val Gly Arg

5

10

15

Ile Val Leu Ser Gly Lys

20

25 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

30 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

35 (ix) FEATURE:

(A) NAME/KEY:

- 28 -

Glu Asp Val Val Cys Cys Acp Cys Val Val Ile Val Gly Arg Ile
5 10 15
Val Leu Ser Gly Lys Lys
20

5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 21
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

15

(ix) FEATURE:

(A) NAME/KEY:

20 Glu Asp Val Val Cys Cys Acp Lys Lys Gly Ser Leu Val Ile Arg
5 10 15
Gly-Val-Ile-Val-Val-Cys
20

(2) INFORMATION FOR SEQ ID NO:4:

25

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
30 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

35

(A) NAME/KEY:

(B) OTHER INFORMATION: Xaa is lysine having a peptide bond
between its ϵ -amino group and the carboxyl group of lysine at position 8.

- 29 -

The carboxyl group of the Xaa forms a peptide bond with the α -amino group of another lysine (not shown);

```

      Glu Asp Val Val Cys Cys Xaa Lys Gly Ser Leu Val Ile Arg Gly
5              5              10              15
      Val Ile Val Val Cys
              20

```

(2) INFORMATION FOR SEQ ID NO:5:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

15

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

(A) NAME/KEY:

(B) OTHER INFORMATION: Amino acid residues at positions 9-21
are preferably D-amino acid residues;

```

      Glu Asp Val Val Cys Cys Acp Acp Lys Gly Ser Leu Val Ile Arg
              5              10              15
25      Gly Val Ile Val Val Cys
              20

```

(2) INFORMATION FOR SEQ ID NO:6:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

35

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- 30 -

(A) NAME/KEY:

(B) OTHER INFORMATION: The lysine residue at position 8 has a peptide bond between the carboxyl group of Acp and the α amino group of the lysine, and the ϵ amino group of the lysine at position 8 forms a peptide bond with the carboxyl group of the cysteine residue at position 9 and the amino acid residues at positions 9-21 are preferably D-amino acid residues;

Glu Asp Val Val Cys Cys AcP Lys Cys Val Val Ile Val Gly Arg
10 5 10 15
Ile Val Leu Ser Gly Lys
 20

(2) INFORMATION FOR SEQ ID NO:7:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

20

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

25

(A) NAME/KEY:

(B) OTHER INFORMATION: Amino acids at positions 8-20 are preferably D-amino acids.

30

| |
|---|
| Glu Asp Val Val Cys Cys Acp Lys Gly Ser Leu Val Ile Arg Gly |
| 5 10 15 |
| Val Ile Val Val Cys Lys |
| 20 |

(2) INFORMATION FOR SEQ ID NO:8:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

- 31 -

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

5

(ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION:

10 Xaa is a lysine wherein the ϵ amino group of which forms a peptide bond with the carboxyl group of the cysteine residue at position 8 and the carboxyl group of the lysine residue forms a peptide bond with an α amino group of another lysine residue (not shown), preferably the amino acid residues at positions 8 - 20 are D- amino acid residues.

15 Glu Asp Val Val Cys Cys Xaa Cys Val Val Ile Val Gly Arg Ile
 5 10 15
Val Leu Ser Gly Lys
 20

20 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

25 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

30 (ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues at positions 1- 13 are preferably D-amino acid residues and lysine at position 14 is preferably an L-amino acid residue;

35

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Lys
5 10

- 32 -

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

10

(ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION: Amino acid residues at positions 1 -
11 are preferably D-amino acids;

15

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Lys

5

10

INFORMATION FOR SEQ ID NO:11:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
25 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

30

(A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues are
preferably D-amino acid residues.

Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly

35

5

10

INFORMATION FOR SEQ ID NO:12:

- 33 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

5 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

10 (A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues are preferably D-amino acids and the serine residue at position 1 is preferably acetylated;

15 Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val

5

INFORMATION FOR SEQ ID NO:13:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

25

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

30 (B) OTHER INFORMATION: The amino acid residues are preferably D-amino acid residues and the lysine residue at position 1 is preferably acetylated.

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys

35

5

10

INFORMATION FOR SEQ ID NO:14:

- 34 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

5 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

10 (A) NAME/KEY:

(B) OTHER INFORMATION: Xaa is biotin and the amino acid residues at positions 2 - 14 are preferably D-amino acids;

Xaa Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Lys

15

5

10

Lys

INFORMATION FOR SEQ ID NO:15:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

25

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

30 (B) OTHER INFORMATION: Xaa is a lysine residue in which the ϵ amino group of the lysine forms a peptide bond with a biotin and amino acid residues at positions 1 - 13 are preferably D-amino acid residues.

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Xaa Lys

35

5

10

15

(2) INFORMATION FOR SEQ ID NO:16:

- 35 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 549 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- 10 (A) NAME/KEY: HCV NS3 Protease

```

GCG CCC ATC ACG GCG TAC GCC CAG CAG ACG AGA GGC CTC CTA GGG 45
Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
1           5           10           15

15 TGT ATA ATC ACC AGC CTG ACT GGC CGG GAC AAA AAC CAA GTG GAG 90
Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
           20           25           30

20 GGT GAG GTC CAG ATC GTG TCA ACT GCT ACC CAA ACC TTC CTG GCA 135
Gly Glu Val Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala
           35           40           45

25 ACG TGC ATC AAT GGG GTA TGC TGG ACT GTC TAC CAC GGG GCC GGA 180
Thr Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly
           50           55           60

ACG AGG ACC ATC GCA TCA CCC AAG GGT CCT GTC ATC CAG ATG TAT 225
30 Thr Arg Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr
           65           70           75

ACC AAT GTG GAC CAA GAC CTT GTG GGC TGG CCC GCT CCT CAA GGT 270
Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly
35           80           85           90

TCC CGC TCA TTG ACA CCC TGC ACC TGC GGC TCC TCG GAC CTT TAC 315
Ser Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr

```

- 36 -

| | | | |
|----|---|-----|-----|
| | 95 | 100 | 105 |
| | CTG GTT ACG AGG CAC GCC GAC GTC ATT CCC GTG CGC CGG CGA GGT 360 | | |
| | Leu Val Thr Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly | | |
| 5 | 110 | 115 | 120 |
| | GAT AGC AGG GGT AGC CTG CTT TCG CCC CGG CCC ATT TCC TAC CTA 405 | | |
| | Asp Ser Arg Gly Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu | | |
| | 125 | 130 | 135 |
| 10 | AAA GGC TCC TCG GGG GGT CCG CTG TTG TGC CCC GCG GGA CAC GCC 450 | | |
| | Lys Gly Ser Ser Gly Gly Pro Leu Leu Cys Pro Ala Gly His Ala | | |
| | 140 | 145 | 150 |
| 15 | GTG GGC CTA TTC AGG GCC GCG GTG TGC ACC CGT GGA GTG ACC AAG 495 | | |
| | Val Gly Leu Phe Arg Ala Ala Val Cys Thr Arg Gly Val Thr Lys | | |
| | 155 | 160 | 165 |
| | GCG GTG GAC TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC ATG AGA 540 | | |
| 20 | Ala Val Asp Phe Ile Pro Val Glu Asn Leu Glu Thr Thr Met Arg | | |
| | 170 | 175 | 180 |
| | TCC CCG GTG | | |
| | Ser Pro Val | | |

25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- | | |
|----|----------------------------|
| | (A) LENGTH: 162 base pairs |
| 30 | (B) TYPE: nucleic acid |
| | (C) STRANDEDNESS: single |
| | (D) TOPOLOGY: linear |

(ii) MOLECULE TYPE: cDNA

35

(ix) FEATURE:

(A) NAME/KEY: NS4A

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```

AGC ACC TGG GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG 45
Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
1           5           10           15

5  TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC 90
Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val
           20           25           30

TTG TCC GGG AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC 135
10 Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr
           35           40           45

CAG GAG TTC GAT GAG ATG GAA GAG TGC 162
Gln Glu Phe Asp Glu Met Glu Glu Cys
15           50

```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: double

25 (ii) MOLECULE TYPE: cDNA

```

GA TCA CCG GTC TAG ATCT
T GGC CAG ATC TAGA

```

30 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 38 -

(ix) FEATURE:

(A) NAME/KEY:

5 CCG GTC CGG AAG AAA AAG AGA CGC TAG C
AG GCC TTC TTT TTC TCT GCG ATC G

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY:

20 CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAA TTC
GT TAA TAT GGA CTG TCC CTC CAA GAG ATG GTC CTT AAG

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC A
CTA CTC TAC CTT CTC ACG GCC TTC TTT TTC TCT GCG TTC GA

25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

30 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

35

(ix) FEATURE:

(A) NAME/KEY: NS4A Active Mutant

- 39 -

Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys
5 10

(2) INFORMATION FOR SEQ ID NO:22:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

15 (A) NAME/KEY: Soluble 5A/5B Substrate

Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr
5 10 15

Gly Lys

20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 810 base pairs

25 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(ix) **FEATURE:**

(A) NAME/KEY: pNB182Δ4AHT

35 ATG AGA GGA TCG CAT CAC CAT CAC CAT CAC ACG GAT CCG CCC ATC 45
Met Arg Gly Ser His His His His His His Thr Asp Pro Pro Ile
1 5 10 15

- 40 -

| | | |
|----|---|-----|
| | ACG GCG TAC GCC CAG CAG ACG AGA GGC CTC CTA GGG TGT ATA ATC | 90 |
| | Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile | |
| | 20 25 30 | |
| 5 | ACC AGC CTG ACT GGC CGG GAC AAA AAC CAA GTG GAG GGT GAG GTC | 135 |
| | Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val | |
| | 35 40 45 | |
| | CAG ATC GTG TCA ACT GCT ACC CAA ACC TTC CTG GCA ACG TGC ATC | 180 |
| 10 | Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr Cys Ile | |
| | 50 55 60 | |
| | AAT GGG GTA TGC TGG ACT GTC TAC CAC GGG GCC GGA ACG AGG ACC | 225 |
| 15 | Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr | |
| | 65 70 75 | |
| | ATC GCA TCA CCC AAG GGT CCT GTC ATC CAG ATG TAT ACC AAT GTG | 270 |
| | Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val | |
| 20 | 80 85 90 | |
| | GAC CAA GAC CTT GTG GGC TGG CCC GCT CCT CAA GGT TCC CGC TCA | 315 |
| | Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser | |
| 25 | 95 100 105 | |
| | TTG ACA CCC TGC ACC TGC GGC TCC TCG GAC CTT TAC CTG GTT ACG | 360 |
| | Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr | |
| 30 | 110 115 120 | |
| | AGG CAC GCC GAC GTC ATT CCC GTG CGC CGG CGA GGT GAT AGC AGG | 405 |
| | Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg | |
| 35 | 125 130 135 | |

- 41 -

GGT AGC CTG CTT TCG CCC CGG CCC ATT TCC TAC CTA AAA GGC TCC 450
 Gly Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser
 140 145 150

5 TCG GGG GGT CCG CTG TTG TGC CCC GCG GGA CAC GCC GTG GGC CTA 495
 Ser Gly Gly Pro Leu Leu Cys Pro Ala Gly His Ala Val Gly Leu
 155 160 165

TTC AGG GCC GCG GTG TGC ACC CGT GGA GTG ACC AAG GCG GTG GAC 540
 10 Phe Arg Ala Ala Val Cys Thr Arg Gly Val Thr Lys Ala Val Asp
 170 175 180

TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC ATG AGA TCC CCG GGG 585
 Phe Ile Pro Val Glu Asn Leu Glu Thr Thr Met Arg Ser Pro Gly
 15 185 190 195

GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG TAT TGC CTG 630
 Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu
 200 205 210

20 TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC TTG TCC GGG 720
 Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly
 215 220 225

25 AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAG TTC 765
 Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu Phe
 230 235 240

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC AAG CTT AAT 810
 30 Asp Glu Met Glu Glu Cys Arg Lys Lys Lys Arg Arg Lys Leu Asn
 245 250 255

(2) INFORMATION FOR SEQ ID NO:24:

- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 162 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

- 42 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5 (ix) FEATURE:

(A) NAME/KEY: Native NS4A

TCA ACA TGG GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG 45
Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
10 1 5 10 15

TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC 90
Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val
20 25 30

15 TTG TCC GGG AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC 135
Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr
35 40 45

20 CAG GAG TTC GAT GAG ATG GAA GAG TGC
Gln Glu Phe Asp Glu Met Glu Glu Cys
50

2) INFORMATION FOR SEQ ID NO:25:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

35

(A) NAME/KEY: Native 5A/5B Substrate

- 43 -

Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr
5 10 15
Gly

5 2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

10 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

15 (ix) FEATURE:

(A) NAME/KEY: NS3/NS4A Cleavage site

Cys Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu
 5 10 15
 20 Val Gly Gly Val Leu
 20

2) INFORMATION FOR SEQ ID NO:27:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: polypeptide

(ix) **FEATURE:**

(A) NAME/KEY: NS4A/4B Cleavage Site

35 Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
5 10 15
Tyr Ile Glu Gln Gly
20

- 44 -

2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY: 4B/5A

15 Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp Leu
5 10 15
Arg Asp Ile Trp Asp
20

20 2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

30 (ix) FEATURE:

(A) NAME/KEY:

Glu-Asp-Val-Val-Cys-Cys-Acp-Lys
5

35

2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- 45 -

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY:

10

Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys

5

10

WE CLAIM:

1. A bivalent inhibitor of an hepatitis C NS3 protease comprised of a
5 first peptide linked to a second peptide, said first peptide being a
subsequence, a mutated subsequence or a mutated full-length sequence
of a substrate of the hepatitis C NS3 protease and said second peptide
being a subsequence of a hepatitis C NS4A polypeptide.
- 10 2. The bivalent inhibitor of claim 1 selected from the group consisting
of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,
SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.
- 15 3. An inhibitor of an HCV protease comprised of a peptide, said peptide
being a subsequence, a mutated subsequence or a mutated full-length
sequence of a substrate of the HCV NS3 protease.
- 20 4. An inhibitor of claim 3 selected from the group consisting of SEQ ID
NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13,
SEQ ID NO: 14 and SEQ ID NO: 15.
- 25 5. An inhibitor of an HCV NS3 protease comprised of a peptide, said
peptide being a subsequence, a mutated subsequence or a mutated full-
length sequence of an HCV NS4A polypeptide.
- 30 6. The use of an inhibitor of an HCV NS3 protease for the manufacture
of a medicament for treating hepatitis C, wherein the inhibitor is
comprised of a first peptide linked to a second peptide, said first peptide
being a subsequence, mutated subsequence or a mutated full-length
sequence of a substrate of the hepatitis C NS3 protease and said second
peptide being a subsequence, a mutated subsequence or a mutated full-
length sequence of a hepatitis C NS4A polypeptide.
- 35 7. The use of claim 6 wherein the inhibitor is selected from the group
consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,
SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

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8. The use of an inhibitor of an HCV NS3 protease for the manufacture of a medicament for treating hepatitis C, wherein the inhibitor is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

9. The use of claim 8 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

10. The use of an inhibitor of an HCV NS3 protease for the manufacture of a medicament for treating hepatitis C, wherein the inhibitor is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV NS4A polypeptide.

11. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being an inhibitor of an HCV NS3 protease, said inhibitor being comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide, and a pharmaceutical carrier.

12. The pharmaceutical composition of claim 11 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

13. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, said inhibitor being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

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14. The pharmaceutical composition of claim 13 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

5

15. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, wherein said inhibitor is comprised of a peptide, said peptide
10 being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV NS4A polypeptide.

15

Figure 1

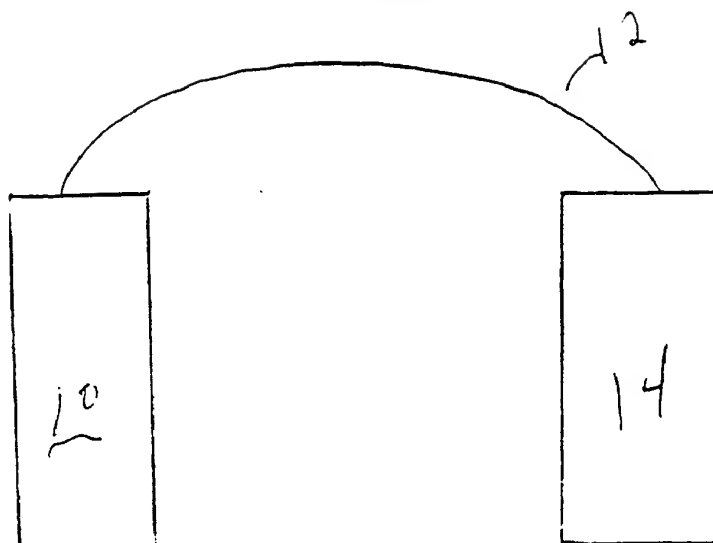


FIGURE 2

1) Construction of the plasmid pBY1015 (Expressing NS3 in E. coli)

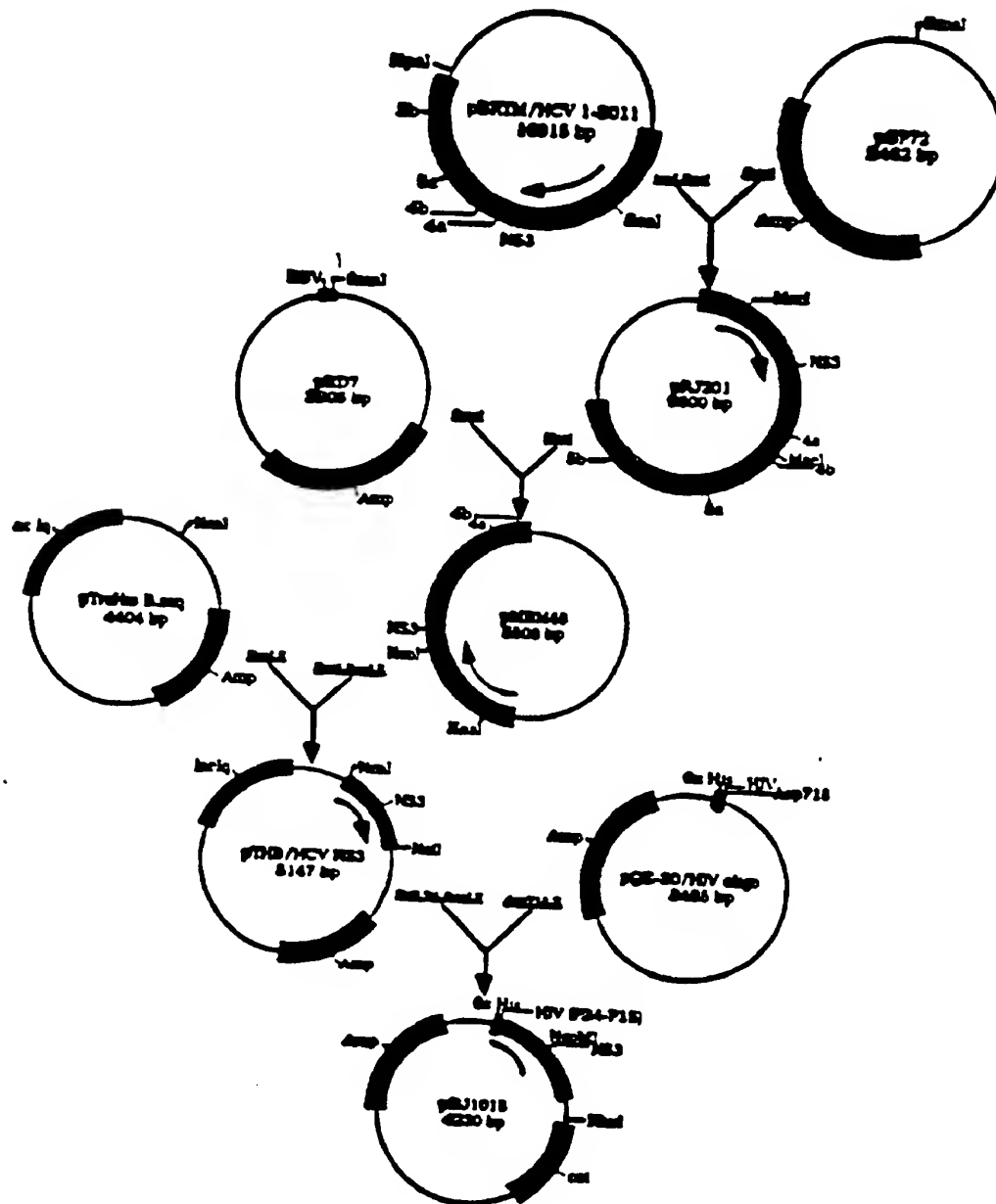


FIGURE 3

ii) Construction of the plasmid pTS56-9 (With a stop codon after aa 183)

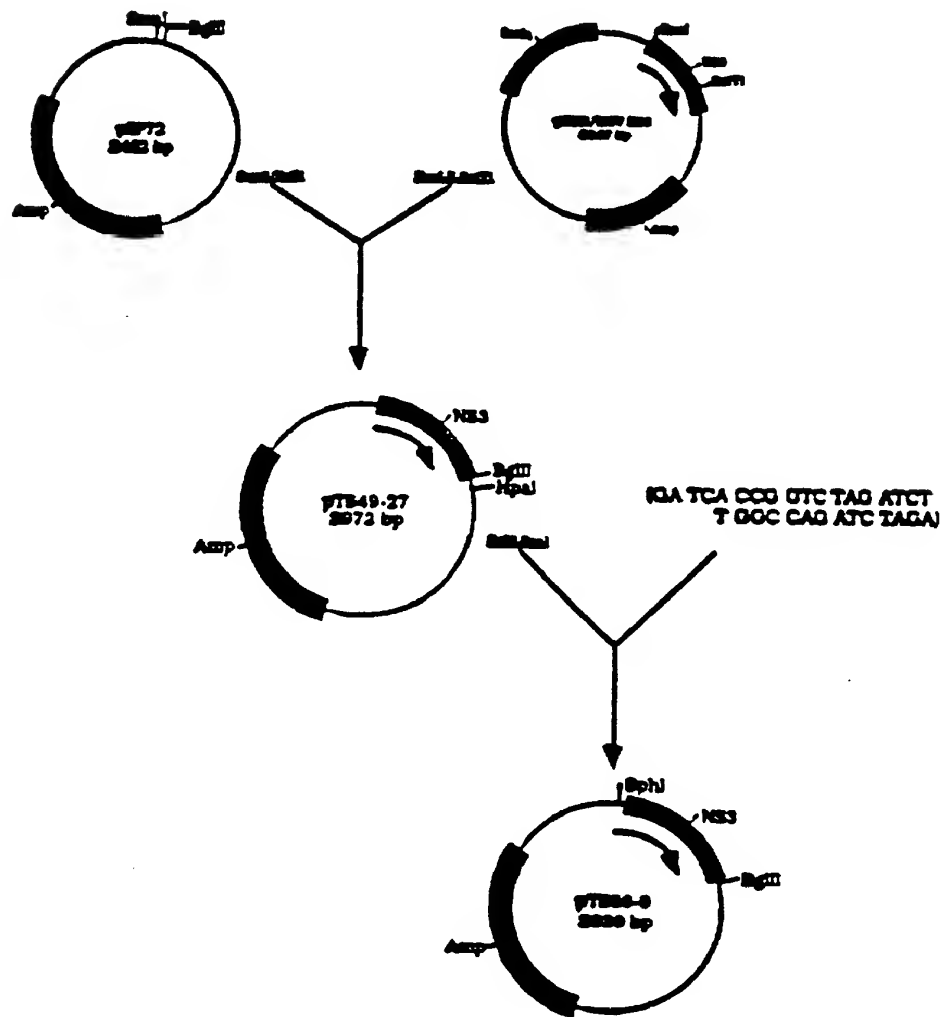


FIGURE 4

22) Construction of the plasmid pJB1006 (Fused with a string of positively charged aa at the carboxy end of NS3 183)

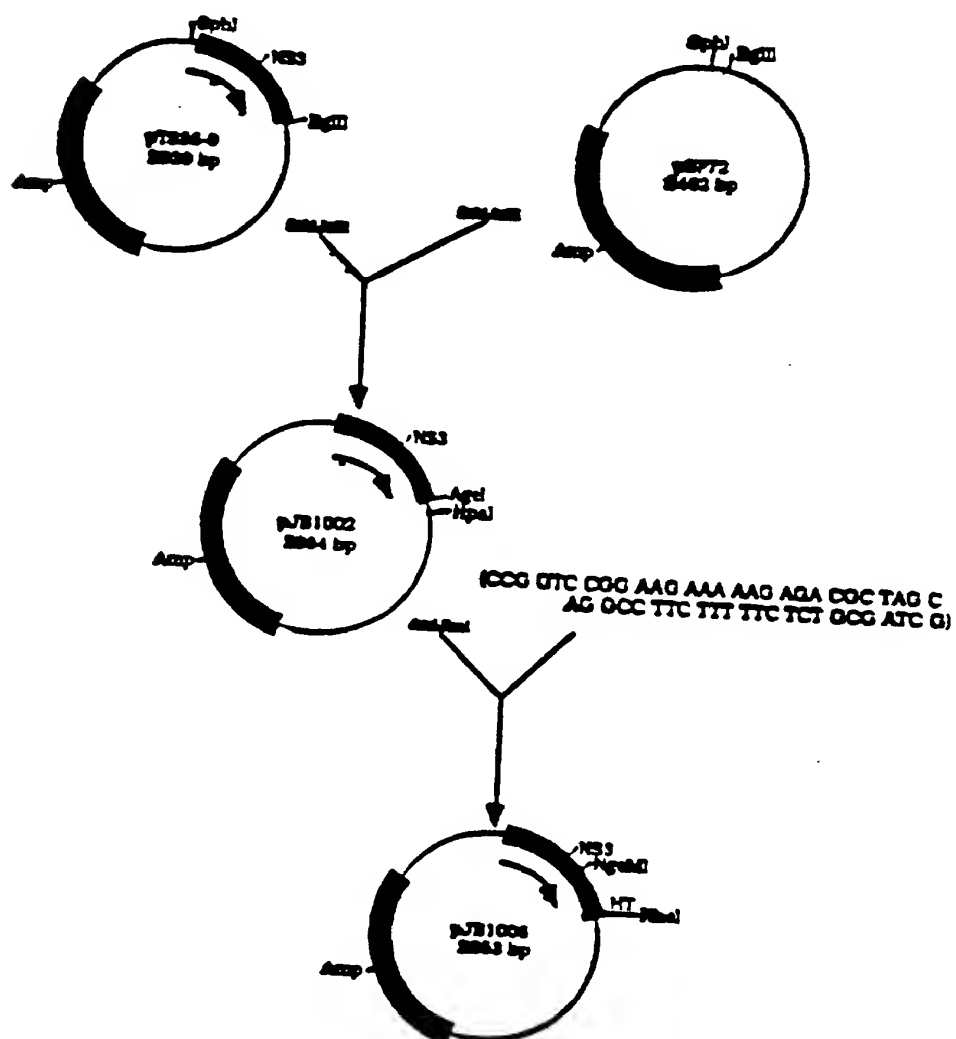
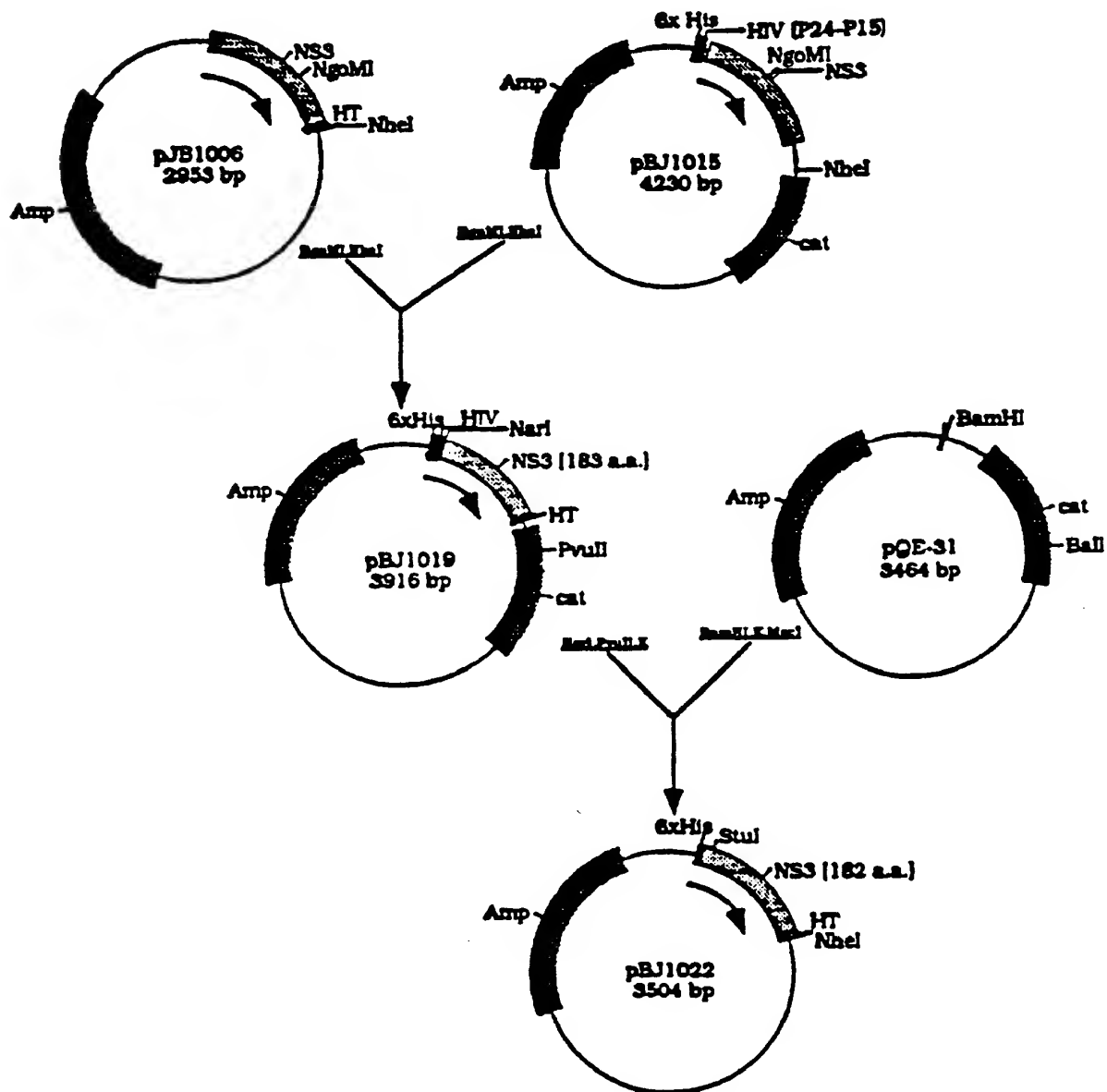


FIGURE 5

iv) Construction of the plasmid pBJ1022 [expressing His-NS3(182)-HT in E.coli]



v) Construction of the plasmid pNB(-V)182-64A HT

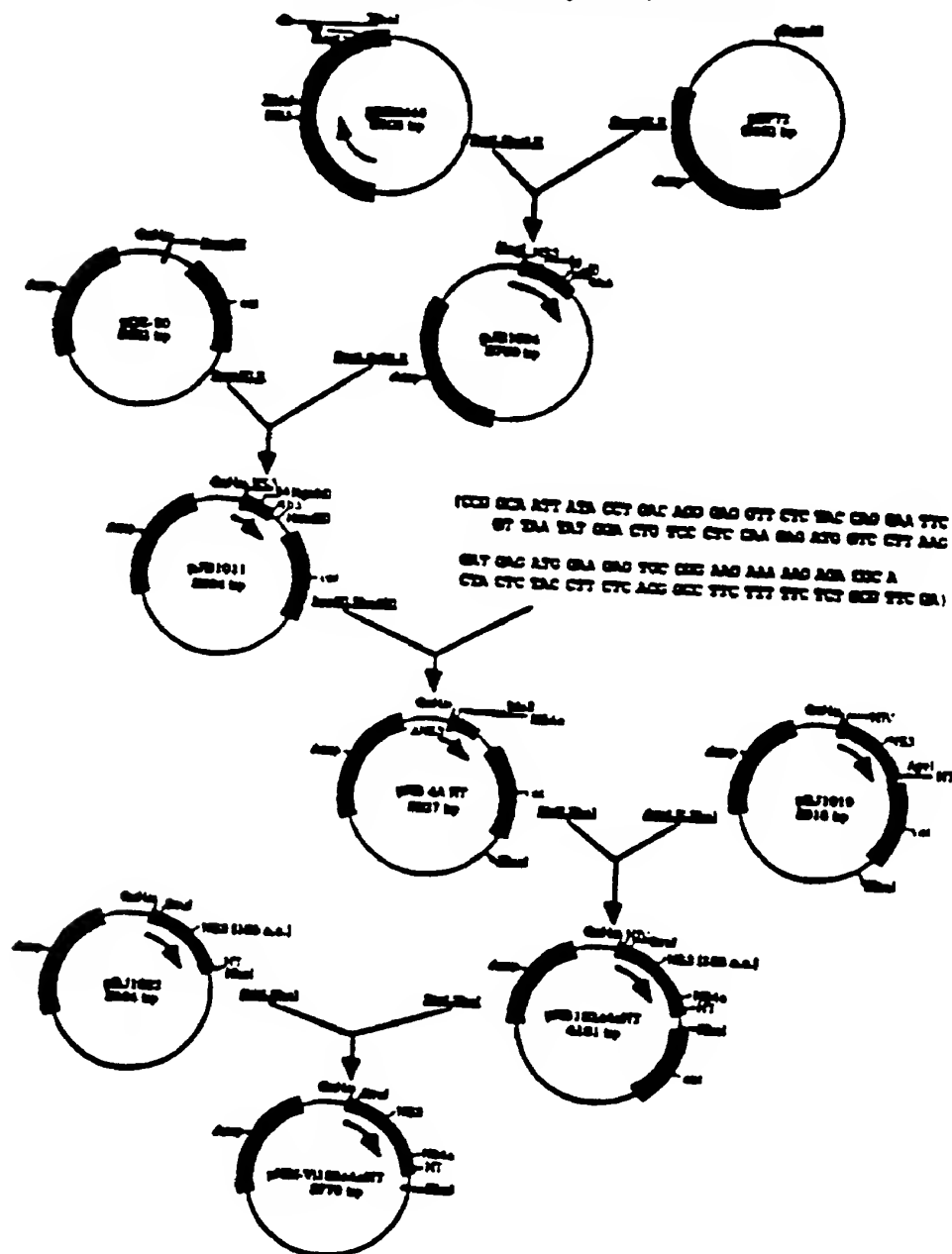
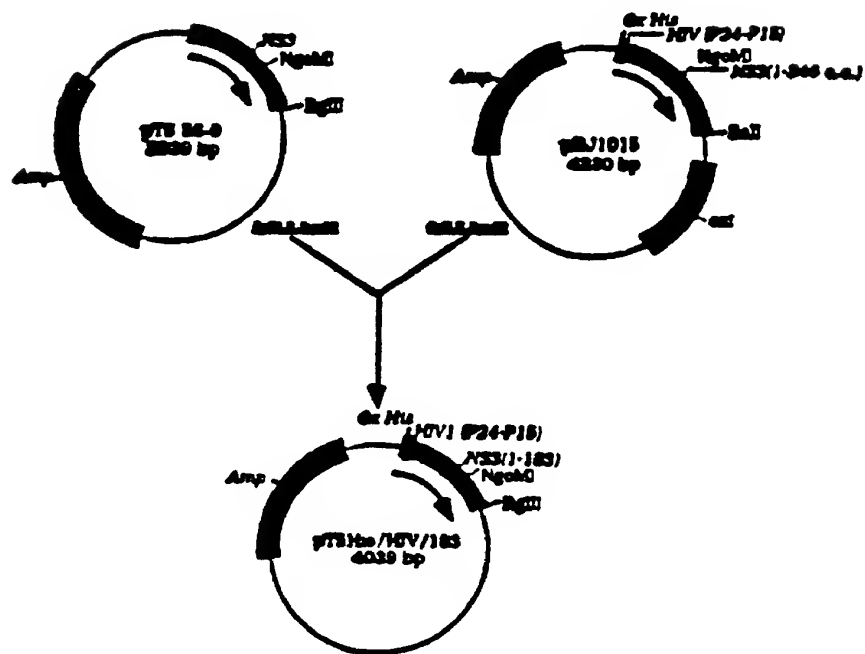


FIGURE 7

Construction of pT5 His/HIV/NS3(183)



INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 97/07632

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K14/18 C07K19/00 A61K39/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| A | WO 95 22985 A (ISTITUTO DI RICERCHE DI BIOLOG ;FRANCESCO RAFFAELE DE (IT); FAILLA) 31 August 1995 see page 3, last paragraph - page 4, paragraph 3; example 4 --- | 1-15 |
| A | HIROAKI OKAMOTO ET AL.: "The 5'-terminal sequence of the Hepatitis C Virus genome " THE JAPANESE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 60, no. 1, January 1990, pages 167-177, XP002042711 see the whole document --- -/-- | 1-15 |

☒ Further documents are listed in the continuation of box C☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

7 October 1997

Date of mailing of the international search report

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International Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| P,X | WO 96 36702 A (SCHERING CORPORATION) 21 November 1996 see page 3, line 15 - line 19 see page 6, line 12 - line 20 see page 13, line 27 - page 14, line 10; example 3 --- | 1,3,6,8, 11,13 |
| P,X | WO 96 35806 A (SCHERING CORPORATION) 14 November 1996 see page 6, line 35 - page 7, line 1; example 5 --- | 3,8,13 |
| P,X | WO 96 35717 A (SCHERING CORPORATION) 14 November 1996 see page 4, line 10 - line 37 see page 13, line 15 - line 37; example 3 ----- | 3,8,13 |

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/07632

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| WO 9522985 A | 31-08-95 | AU 1822395 A | 11-09-95 |
| | | CA 2182521 A | 31-08-95 |
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| WO 9635717 A | 14-11-96 | AU 5729296 A | 29-11-96 |
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| (21) International Application Number: PCT/US97/07632 (22) International Filing Date: 8 May 1997 (08.05.97) (30) Priority Data: 08/644,544 10 May 1996 (10.05.96) US (71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US). (72) Inventors: ZHANG, Rumin; 4 Devon Road, Edison, NJ 08820 (US). MUI, Philip, W.; 1 Windswept Lane, Freehold, NJ 07728 (US). WEBER, Patricia, C.; 1970 Timber Lakes Drive, Yardley, PA 19067 (US). (74) Agents: DULAK, Norman, C. et al.; Schering-Plough Corporation, Patent Dept. K-6-1 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US). | | (81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, HU, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: SYNTHETIC INHIBITORS OF HEPATITIS C VIRUS NS3 PROTEASE (57) Abstract An inhibitor of the HCV NS3 protease. The inhibitor is a subsequence of a substrate of the NS3 protease or a subsequence of the NS4A cofactor. Another inhibitor of the present invention contains a subsequence of a substrate linked to a subsequences of the NS4A cofactor. In another embodiment the inhibitor is a bivalent inhibitor comprised of a subsequence, a mutated subsequence or a mutated full-length of a substrate of the NS3 protease linked to a subsequence, a mutated subsequence or a mutated full-length subsequence of the HCV NS4A cofactor. | | |

*(Referred to in PCT Gazette No. 28/1998, Section II)

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SYNTHETIC INHIBITORS OF HEPATITIS C VIRUS NS3 PROTEASE

5

BACKGROUND OF THE INVENTION

10 Hepatitis C virus (HCV) is considered to be the major etiological agent of non-A non-B (NANB) hepatitis, chronic liver disease, and hepatocellular carcinoma (HCC) around the world. The viral infection accounts for greater than 90% of transfusion -associated hepatitis in U.S. and it is the predominant form of hepatitis in adults over 40 years of age. Almost all of the infections result in chronic hepatitis and nearly 20% develop liver cirrhosis.

20 The virus particle has not been identified due to the lack of an efficient *in vitro* replication system and the extremely low amount of HCV particles in infected liver tissues or blood. However, molecular cloning of the viral genome has been accomplished by isolating the messenger RNA (mRNA) from the serum of infected chimpanzees then cloned using recombinant methodologies. [Grakoui A. *et al. J. Virol.* 67: 1385 - 1395 (1993)] It is now known that HCV contains a positive strand RNA genome comprising approximately 9400 nucleotides, whose organization is similar to that of flaviviruses and pestiviruses. The genome of HCV, like that of flavi- and pestiviruses, encodes a single large polyprotein of about 3000 amino acids which undergoes proteolysis to form mature viral proteins in infected cells.

30

Cell-free translation of the viral polyprotein and cell culture expression studies have established that the HCV polyprotein is processed by cellular and viral proteases to produce the putative structural and nonstructural (NS) proteins. At least nine mature viral proteins are produced from the polyprotein by specific proteolysis. The order and nomenclature of the cleavage products are as follows: NH₂-C-E1-E2-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. The three amino terminal putative structural proteins, C (capsid), E1, and E2 (two

- 2 -

envelope glycoproteins), are believed to be cleaved by host signal peptidases of the endoplasmic reticulum(ER) . The host enzyme is also responsible for generating the amino terminus of NS2 . The proteolytic processing of the nonstructural proteins are carried out by the viral proteases: NS2-3 and NS3, contained within the viral polyprotein. The NS2-3 protease catalyzes the cleavage between NS2 and NS3. It is a metalloprotease and requires both NS2 and the protease domain of NS3. The NS3 protease catalyzes the rest of the cleavages of the substrates in the nonstructural part of the polyprotein. The NS3 protein contains 631 amino acid residues and is comprised of two enzymatic domains: the protease domain contained within amino acid residues 1-181 and a helicase ATPase domain contained within the rest of the protein. It is not known if the 70 kD NS3 protein is cleaved further in infected cells to separate the protease domain from the helicase domain, however, no cleavage has been observed in cell culture expression studies.

The NS3 protease is a member of the serine proteinase class of enzymes. It contains His, Asp, and Ser as the catalytic triad. Mutation of the catalytic triad residues abolishes the cleavages at substrates NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B. The cleavage between NS3 and NS4A is mediated through an intramolecular enzymatic reaction, whereas the cleavages at NS4A/4B, 4B/5A, 5A/5B sites occur in a *trans* enzymatic reaction.

Experiments using transient expression of various forms of HCV NS polyproteins in mammalian cells have established that the NS3 serine protease is necessary but not sufficient for efficient processing of all these cleavages. Like flaviviruses, the HCV NS3 protease also requires a cofactor to catalyze some of these cleavage reactions. In addition to the serine protease NS3, the NS4A protein is absolutely required for the cleavage of the substrate at the NS3/4A and 4B/5A sites and increases the efficiency of cleavage of the substrate between 5A/5B, and possibly 4A/4B.

Because the HCV NS3 protease cleaves the non-structural HCV proteins which are necessary for the HCV replication, the NS3 protease can be a target for the development of therapeutic agents against the

HCV virus. Thus there is a need for the development of inhibitors of the HCV protease.

SUMMARY OF THE INVENTION

5

The present invention fills this need by providing for a bivalent inhibitor of an hepatitis C NS3 protease comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide.

15 The present application further provides for an inhibitor of an HCV protease comprised of a peptide, said peptide being a subsequence, a mutated subsequence, or a mutated full-length sequence of a substrate of the HCV NS3 protease.

20 The present application further provides for an inhibitor of an HCV NS3 protease comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of an HCV NS4A polypeptide.

25 The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor being comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide.

35 The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor being comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

The present invention further comprises a method for treating an individual infected with the HCV virus comprising administering an inhibitor of an HCV NS3 protease to said individual, said inhibitor
5 being comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of an HCV NS4A polypeptide.

The present invention further comprises a pharmaceutical
10 composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being an inhibitor of an HCV NS3 protease, said inhibitor being comprised of a first peptide linked to a second peptide, said first peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the
15 hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide, and a pharmaceutical carrier.

The present invention further provides for a pharmaceutical
20 composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, said inhibitor being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

25
The present invention further provides for a pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, wherein said inhibitor
30 is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV NS4A polypeptide.

35

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 schematically depicts an embodiment of a bivalent inhibitor of the present invention.

5

Figure 2 depicts the recombinant synthesis of plasmid pBJ1015.

Figure 3 depicts the recombinant synthesis of plasmid pTS56-9.

10

Figure 4 depicts the recombinant synthesis of plasmid pJB1006.

Figure 5 depicts the recombinant synthesis of plasmid pBJ1022.

15

Figure 6 depicts the recombinant synthesis of plasmid pNB(-V)182Δ4AHT.

Figure 7 depicts the recombinant synthesis of plasmid pT5His/HIV/183.

DETAILED DESCRIPTION OF THE INVENTION

20

The teachings of all references cited are incorporated herein in their entirety by reference.

The present invention are inhibitors of the HCV NS3 protease.

25 The present invention relates to inhibitors of the HCV NS3 protease which inhibit either the interaction of a substrate or cofactor NS4A with the NS3 protease or a bivalent inhibitor which inhibits the interaction of the NS3 protease with both cofactor NS4A and a substrate of the NS3 protease. Compared to inhibitors targeting only at a single binding site,

30 bivalent enzyme inhibitors may provide additional advantages in terms of higher binding affinity (potency), as well as enhanced specificity against similar cellular host enzymes for reduced toxicity effects.

Design Strategy of Bivalent Inhibitors of HCV NS3 Protease

35

The basic strategy for the design of bivalent inhibitors of HCV NS3 protease involved the devise of a molecular framework consisting of three individual components:

- 6 -

1. a region appropriate for binding to a substrate binding site;
 2. a region suitable for binding to the NS4A binding site;
 3. a flexible linker region connecting regions (1) and (2) which
- 5 would allow the two end regions to bind to their respective binding sites.

Schematically, this is represented by Figure 1 in which the substrate subsequence is depicted as block, 10, being attached to linker 12, and said linker 12 being attached to the polypeptide NS4A designated 14.

Since the NS3 protease cleaves the HCV polyprotein at the NS3/4A, 4A/4B, 4B/5A and 5A/5B junctions, then subsequences of or mutated subsequences of these sites can be used as substrate inhibitors.

15 A substrate inhibitor which is a subsequence of the inhibitor should be a subsequence which is prior to or after the cleavage site but preferably should not contain the cleavage site. A mutated subsequence or mutated full-length sequence of the substrate can be used if the cleavage site is mutated so that the cleavage of the substrate does not occur

20 leads to mechanism-based inactivation of the protease.

For example, the NS3/4A cleavage site contains the following sequence:

25 Cys Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu
5 10 15
Val Gly Gly Val Leu (SEQ. ID NO.: 26)
20

The cleavage site is between the threonine at position 10 and the serine at position 11. Any subsequence inhibitor should preferably be before the serine or after the threonine residue. Alternatively, a mutated subsequence or sequence can be produced by changing the threonine/serine cleavage site at position 10-11 to eliminate the cleavage site.

35

- 7 -

NS4A/4B contains the following sequence.

```

      Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
                    5                      10                      15
5      Tyr Ile Glu Gln Gly (SEQ ID NO.: 27).
                    20

```

The cleavage site is between the cysteine residue at position 10 and the serine at position 11. Any subsequence should preferably be before the serine or after the cysteine, but should preferably not contain both the cysteine and the serine. Alternatively, a mutated subsequence or sequence can be produced by changing the cysteine/serine cleavage site at position 10 - 11 to eliminate the cleavage site.

NS4B/5A contains the following sequence.

```

      Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp Leu
                    5                      10                      15
20     Arg Asp Ile Trp Asp (SEQ ID NO.: 28)
                    20

```

The cleavage site is between the cysteine at position 10 and serine at position 11. Any subsequence should preferably end before the serine or start after the cysteine but should preferably not contain both the serine and the cysteine. Alternatively, a mutated subsequence or sequence can be produced by changing the cysteine/serine cleavage sit at position 10 - 11 to eliminate the cleavage site.

NS5A/5B contains the following sequence.

```

30     Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr
                    5                      10                      15
      Gly (SEQ. ID NO.: 25)

```

The cleavage site is between the cysteine at position 8 and the serine at position 9. Any subsequence should preferably end at the cysteine or start at the serine, but should preferably not contain both the cysteine and the serine. Alternatively, a mutated sequence or subsequence can be

- 8 -

produced by changing the cysteine/serine cleavage site at position 8 - 9 to eliminate the cleavage site.

5 Linker 12 can be any chemical entity that can form a bond with polypeptides 10 and 14. Preferably the linker should be equivalent in length to a carbon chain having about 7-14 carbon residues. Examples of suitable linkers are two 6-aminocaproic acid (Acp) residues or an Acp and Lys wherein one of the polypeptides 10 or 14 form a peptide bond
10 with the ϵ amine of lysine.

Examples of bivalent inhibitors of the present invention are the following:

15 Glu-Asp-Val-Val-Cys-Cys-Acp-Acp-Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 1)

 Glu-Asp-Val-Val-Cys-Cys-Acp-Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys-Lys (SEQ ID NO:2)

20 Glu-Asp-Val-Val-Cys-Cys-Acp-Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 3)

 wherein Xaa is a lysine residue having a peptide bond between its
25 ϵ -amino and the carboxyl group of the following lysine which forms a peptide bond with the glycine at position 10. Furthermore, the glutamic acid residue at position 1 may or may not be acetylated.

30 Glu-Asp-Val-Val-Cys-Cys-Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 4)

 wherein Xaa is Lysine having a peptide bond between its ϵ -amino and the carboxyl group of the following lysine which forms a peptide bond with the Gly; furthermore, the carboxyl group of the Xaa forms a
35 peptide bond with the α -amino group of another lysine (not shown);

 Glu-Asp-Val-Val-Cys-Cys-Acp-Acp-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys (SEQ ID NO: 5)

- 9 -

wherein the amino acids at positions 9-21 are preferably D-amino acids;

5 Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-Cys-Val-Val-Ile-
Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 6)

wherein the lysine residue at position 8 has a peptide bond
between the carboxyl of Acp and the α amino group of the lysine, and
10 the ϵ amino group of the lysine at position 8 forms a peptide bond with
the carboxyl group of the cysteine residue at position 9 and the amino
acid residues at positions 9-21 are preferably D-amino acid residues;

Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-Gly-Ser-Leu-Val-Ile-Arg-
15 Gly-Val-Ile-Val-Val-Cys-Lys (SEQ ID NO: 7)

wherein amino acid residues at positions 8-20 are preferably D-amino acid residues;

20 Glu-Asp-Val-Val-Cys-Cys-Xaa-Cys-Val-Val-Ile-Val-Gly-
Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO: 8)

wherein Xaa is a Lys which forms a peptide bond between its ϵ -
amino acid and the carboxyl group of the Cys residue at position 8 and
25 the carboxyl group of the Lys residue forms a peptide bond with an alpha
amino group of another Lys residue (not shown), preferably the amino
acid residues at positions 8 - 20 are D- amino acids.

Examples of suitable monovalent inhibitors of the present
30 invention are the following:

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys-Lys
(SEQ ID NO.: 9)

wherein the amino acid residues at positions 1- 13 are preferably
35 D-amino acid residues;

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Lys (SEQ ID NO.: 10)

wherein amino acid residues at positions 1 - 11 are preferably
D-amino acid residues;

Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys
(SEQ ID NO.: 11)

- 5 wherein the amino acid residues are preferably D-amino acid residues;

Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val (SEQ ID NO.: 12)

- 10 wherein the amino acid residues are preferably D-amino acid residues and the serine residue at position 1 has been preferably acetylated;

Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys
(SEQ ID NO.: 13)

- 15 wherein the amino acid residues are preferably D-amino acid residues the lysine residue at position 1 is preferably acetylated;

- 20 Xaa-Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile Val-Val-Cys-Lys-Lys
(SEQ ID NO.: 14);

 wherein Xaa is biotin and the amino acid residues at positions 2 - 14 are preferably D-amino acid residues;

- 25 Lys-Gly-Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val-Val-Cys-Xaa-Lys
(SEQ ID NO.: 15);

- 30 Xaa is a lysine residue in which the ϵ amino group of the lysine forms a peptide bond with a biotin, and amino acid residues at positions 1 - 13 are preferably D-amino acid residues.

- 35 The inhibitors of the present invention can be synthesized by a suitable method such as by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis.
The polypeptides are preferably prepared by solid phase peptide synthesis as described by Merrifield, J. Am. Chem. Soc. 85:2149 (1963). The synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-

chains are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the polypeptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The
5 conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise polypeptide synthesis. Included are
10 acyl type protecting groups (*e.g.*, formyl, trifluoroacetyl, acetyl), aryl type protecting groups (*e.g.*, biotiny), aromatic urethane type protecting groups [*e.g.*, benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxy-carbonyl (Fmoc)],
15 aliphatic urethane protecting groups [*e.g.*, t-butyloxycarbonyl (tBoc), isopropylloxycarbonyl, cyclohexyloxycarbonyl] and alkyl type protecting groups (*e.g.*, benzyl, triphenylmethyl). The preferred protecting groups are tBoc and Fmoc, thus the peptides are said to be synthesized by tBoc and Fmoc chemistry, respectively.

20 The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction
25 conditions that will not alter the finished polypeptide. In tBoc chemistry, the side-chain protecting groups for trifunctional amino acids are mostly benzyl based. In Fmoc chemistry, they are mostly tert.-butyl or trityl based.

30 In tBoc chemistry, the preferred side-chain protecting groups are tosyl for Arg, cyclohexyl for Asp, 4-methylbenzyl (and acetamidomethyl) for Cys, benzyl for Glu, Ser and Thr, benzyloxymethyl (and dinitrophenyl) for His, 2-Cl-benzyloxycarbonyl for Lys, formyl for Trp and 2-bromobenzyl for Tyr. In Fmoc
35 chemistry, the preferred side-chain protecting groups are 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg, trityl for

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Asn, Cys, Gln and His, tert-butyl for Asp, Glu, Ser, Thr and Tyr, tBoc for Lys and Trp.

5 Solid phase synthesis is usually carried out from the carboxyl-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl, chlorotriyl or hydroxymethyl resin, and the resulting polypeptide will have a
10 free carboxyl group at the C-terminus. Alternatively, when an amide resin such as benzhydrylamine or p-methylbenzhydrylamine resin (for tBoc chemistry) and Rink amide or PAL resin (for Fmoc chemistry) is used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These
15 resins, whether polystyrene- or polyamide-based or polyethyleneglycol-grafted, with or without a handle or linker, with or without the first amino acid attached, are commercially available, and their preparations have been described by Stewart et al (1984)., "Solid Phase Peptide Synthesis" (2nd Edition), Pierce Chemical Co.,
20 Rockford, IL.; and Bayer & Rapp (1986) *Chem. Pept. Prot.* 3, 3; and Atherton, et al. (1989) *Solid Phase Peptide Synthesis: A Practical Approach*, IRL Press, Oxford.

The C-terminal amino acid, protected at the side-chain if
25 necessary and at the alpha-amino group, is attached to a hydroxymethyl resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIPCDI) and carbonyldiimidazole (CDI). It can be attached to chloromethyl or chlorotriyl resin directly in its cesium
30 tetramethylammonium salt form or in the presence of triethylamine (TEA) or diisopropylethylamine (DIEA). First amino acid attachment to an amide resin is the same as amide bond formation during coupling reactions

35 Following the attachment to the resin support, the alpha-amino protecting group is removed using various reagents depending on the protecting chemistry (e.g., tBoc, Fmoc). The extent of Fmoc removal can be monitored at 300-320 nm or by a

- 13 -

conductivity cell. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

5 Various activating agents can be used for the coupling reactions including DCC, DIPCDI, 2-chloro-1,3-dimethylimidium hexafluorophosphate (CIP), benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and its pyrrolidine analog (PyBOP), bromo-tris-pyrrolidino-phosphonium
10 hexafluorophosphate (PyBroP), N- [(1H-benzotriazol-1-yl) - (dimethylamino) methylene] -N-methylmethanaminium hexafluorophosphate N-oxide (HBTU) and its tetrafluoroborate analog (TBTU) or its pyrrolidine analog (HBPYU), (HATU) and its tetrafluoroborate analog (TATU) or pyrrolidine analog (HAPYU). The
15 most common catalytic additives used in coupling reactions include 4-dimethylaminopyridine (DMAP), 3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine (HODhbt), N-hydroxybenzotriazole (HOBT) and 1-hydroxy-7-azabenzotriazole (HOAt). Amino acid fluorides or chlorides may be used for difficult couplings. Each protected amino
20 acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling reaction can be monitored at each stage, *e.g.*, by the ninhydrin reaction as described by Kaiser *et al.*, *Anal. Biochem.* 34:595 (1970). In cases
25 where incomplete coupling is found, the coupling reaction is extended and repeated and may have chaotropic salts added. The coupling reactions can be performed automatically with commercially available instruments such as ABI model 430A, 431A and 433A peptide synthesizers.

30

After the entire assembly of the desired peptide, the peptide-resin is cleaved with a reagent with proper scavengers. The Fmoc peptides are usually cleaved and deprotected by TFA with scavengers (*e.g.*, H₂O, ethanedithiol, phenol and thioanisole). The tBoc peptides
35 are usually cleaved and deprotected with liquid HF for 1-2 hours at -5 to 0°C, which cleaves the polypeptide from the resin and removes most of the side-chain protecting groups. Scavengers such as anisole, dimethylsulfide and p-thiocresol are usually used with the liquid HF

to prevent cations formed during the cleavage from alkylating and acylating the amino acid residues present in the polypeptide. The formyl group of Trp and dinitrophenyl group of His need to be removed, respectively, by piperidine and thiophenol in DMF prior to the HF cleavage. The acetamidomethyl group of Cys can be removed by mercury(II) acetate and alternatively by iodine, thallium (III) trifluoroacetate or silver tetrafluoroborate which simultaneously oxidize cysteine to cystine. Other strong acids used for tBoc peptide cleavage and deprotection include trifluoromethanesulfonic acid (TFMSA) and trimethylsilyltrifluoroacetate (TMSOTf).

In particular the peptides of the present invention were assembled from a Fmoc-Amide resin or a Fmoc-L-Lys- (tBoc) - Wang resin on an ABI model 433A synthesizer (Applied Biosystems, Foster City, CA) by solid phase peptide synthesis method as originally described by Merrifield, J. Am.Chem.Soc. 85:2149 (1963) but with Fmoc chemistry. The side chains of trifunctional amino acids were protected by tert.-butyl for Glu, Asp and Ser, trityl for Cys, tert.-butyloxycarbonyl (tBoc) for Lys and 2,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for Arg. N-a-Fmoc protected amino acids were pre-activated by HATU and 1-hydroxy-7-azabenzotriazole (HOAt) prior to coupling to the resin. Dimethylsulfoxide (20%) was added during conditional extended coupling and Fmoc deprotection reactions. The synthesis of the inhibitors SEQ ID NOs: 1, 2, 5, 7, and 9-15 was accomplished by sequential and linear assembly of appropriate D- and L-amino acids and achiral amino acids (Gly and Ahx). The synthesis of the inhibitors SEQ ID NOs: 3, 4, 6, and 8 required orthogonal chain assembly anchored at a Lys residue whose side chain amino group was protected by 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-ethyl (Dde). For example, for the preparation of the inhibitor SEQ ID NO: 3, Ac-Glu-Asp-Val-Val-Cys-Cys-Acp-Lys-(Amide resin) (SEQ ID NO: 29) was first assembled. Then the Dde protecting group on the Lys residue was removed by 2% hydrazine in dimethylformamide (Bycroft, B.W. et al J. Chem. Soc. Chem. Commun. 1993, 778). Finally the second arm Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys (SEQ ID NO:30) was sequentially assembled from the side chain amino group. The assembled peptide was cleaved from the resin with simultaneous

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deprotection of side chain protecting groups for three hours by trifluoroacetic acid (TFA) with proper scavengers (80% TFA : 4% phenol : 4% H₂O, 4% thioanisole : 4% ethanedithiol : 4% triisopropylsilane).

The cleaved peptide was separated from the resin by filtration and precipitated and repeatedly washed in anhydrous ethyl ether. The precipitated peptide was lyophilized in H₂O overnight. The lyophilized crude peptide was purified by reverse phase HPLC. The purified peptide was further analyzed by HPLC, mass spectroscopy and amino acid analysis.

10

One can ascertain if a potential compound is effective as an inhibitor of the HCV NS3 protease by using a high throughput assay utilizing the NS3 protease, the NS4 cofactor and the peptide substrates, either 4B/5A or 5A/5B. These can be used to screen for compounds which inhibit proteolytic activity of the protease. One does this by developing techniques for determining whether or not a compound will inhibit the NS3 protease from cleaving the viral substrates. If the substrates are not cleaved, the virus cannot replicate. One example of such a high throughput assay is the scintillation proximity assay (SPA). SPA technology involves the use of beads coated with scintillant. Bound to the beads are acceptor molecules such as antibodies, receptors or enzyme substrates which interact with ligands or enzymes in a reversible manner.

25

For a typical SPA based protease assay the substrate peptide is biotinylated at one end and the other end is radiolabelled with low energy emitters such as ¹²⁵I or ³H. The labeled substrate is then incubated with the enzyme. Avidin coated SPA beads are then added which bind to the biotin. When the substrate peptide is cleaved by the protease, the radioactive emitter is no longer in proximity to the scintillant bead and no light emission takes place. Inhibitors of the protease will leave the substrate intact and can be identified by the resulting light emission which takes place in their presence.

35

Another example of a suitable assay technique is an HPLC assay in which the resultant reaction mixture containing the NS3 protease, the substrate products and the potential inhibitor is resolved on an HPLC column to determine the extent of the cleavage of the substrate. If the

substrate has not been cleaved or the cleavage has been inhibited, then only the intact substrate would be present or a reduced amount of the cleaved product will be shown to be present. If this is the case, then the compound is an effective inhibitor of the NS3 protease.

5

Pharmaceutical Compositions

The dosage level of inhibitors necessary for effective therapy to inhibit the HCV NS3 protease will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents.

Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. See also Langer (1990) Science 249:1527-1533. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. 1 μ g per kilogram weight of the patient to 500 mg per kilogram weight of the patient with an appropriate carrier is a range from which the dosage can be chosen. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

The inhibitors of the HCV NS3 protease of the present invention may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is

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preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press, Parrytown, NY; Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds.)(1993) Pharmaceutical Dosage Forms: Parenteral Medications 2d ed., Dekker, NY; Lieberman, et al. (eds.)(1990) Pharmaceutical Dosage Forms: Tablets 2d ed., Dekker, NY; and Lieberman, et al. (eds.)(1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

The following examples are included to illustrate but not to limit the present invention.

Example 1

Bivalent Inhibitors of HCV NS3 Protease

The bivalent inhibitors of defined by SEQ ID NOs.: 1-10 were synthetically produced as described above and tested for their ability to inhibit the HCV NS3 protease as follows.

Into an aqueous solution containing 25 mM TRIS, 50 mM NaCl, .5 mM EDTA, 10% glycerol and .1% NP40 was placed the potential inhibitor, the HCV NS3 protease at a concentration of 0.05 μ M - 0.1 mM, the HCV NS4A cofactor at a concentration of 0.05 μ M - 0.1 μ M and the 5A/5B substrate at a concentration of 50 μ M. This solution was then incubated for approximately 2 hours at 30°C after which the solution was applied to an HPLC to determine if the 5A/5B remained intact and

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thus the compound was determined to be an inhibitor. However, if the HPLC showed that 5A and 5B were present without the 5A/5B then the compound is not an inhibitor. The potential inhibitors were assayed at several different concentrations to determine the concentration which produced 50% inhibition of the HCV NS3 protease. The results are shown below.

| | <u>Inhibitor</u> | <u>IC₅₀ (μM)</u> |
|----|------------------|-----------------------------|
| | SEQ ID NO:1 | 0.6 |
| | 50571-120 | |
| 10 | SEQ ID NO:2 | 3.0 |
| | 50962-13 | |
| | SEQ ID NO:3 | 3.0 |
| | 50828-001 | |
| | SEQ ID NO:4 | 3 - 30 |
| 15 | 50962-22 | |
| | SEQ ID NO:5 | 0.2 |
| | 50571-144 | |
| | SEQ ID NO:6 | 2.0 |
| | 50571-150 | |
| 20 | SEQ ID NO:7 | 0.2 |
| | 50828-131 | |
| | SEQ ID NO:8 | 0.2 |
| | 50962-24 | |

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Example 2Monovalent Inhibitors of the HCV NS3 Protease

5 Examples of monovalent inhibitors of the HCV NS3 protease are as follows.

| | Inhibitor | <u>IC₅₀(μM)</u> |
|----|----------------|---|
| 10 | SEQ ID NO.: 9 | 0.2 |
| | 50828-129 | |
| | SEQ ID NO.: 10 | 5 |
| | 50962-004 | |
| | SEQ ID NO.: 11 | 0.2 |
| 15 | 50828-70 | |
| | SEQ ID NO.: 12 | 0.6 |
| | 50828-116 | |
| | SEQ ID NO.: 13 | 2.0 |
| | 50571-147 | |
| 20 | SEQ ID NO.: 14 | 0.4 |
| | 50962-047 | |
| | SEQ ID NO.: 15 | 0.4 |
| | 50962-050 | |

25

Examples 3

30

Production of HCV NS3 Protease

A. Plasmid constructions.

35 Several plasmids were designed and constructed using standard recombinant DNA techniques (Sambrook, Fritsch & Maniatis) to express the HCV protease in *E. coli* (Fig 2-7). All HCV specific sequences originated from the parental plasmid pBRTM/HCV 1-3011 (Grakoui *et*

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al.1993). To express the N-terminal 183 amino acid versions of the protease, a stop codon was inserted into the HCV genome using synthetic oligonucleotides (Fig. 3). The plasmids designed to express the N-terminal 246 amino acid residues were generated by the natural NcoI restriction site at the C-terminus.

i) Construction of the plasmid pBJ1015 (Figure 2)

The plasmid pBRTM/HCV 1-3011 containing the entire HCV genome (Grakoui A., *et al.*, *J. Virol.* 67: 1385-1395) was digested with the restriction enzymes Sca I and Hpa I and the 7138 bp (base pair) DNA fragment was isolated and cloned to the Sma I site of pSP72 (Promega) to produce the plasmid, pRJ201. The plasmid pRJ 201 was digested with Msc I and the 2106 bp Msc I fragment was isolated and cloned into the Sma I site of the plasmid pBD7. The resulting plasmid pMBM48 was digested with Kas I and Nco I, and the 734 bp DNA fragment after blunt ending with Klenow polymerase was isolated and cloned into Nco I digested, klenow polymerase treated pTrc HIS B seq expression plasmid (Invitrogen). The ligation regenerated a Nco I site at the 5' end and Nsi I site at the 3' end of HCV sequence. The plasmid pTHB HCV NS3 was then digested with Nco I and Nsi I, and treated with klenow polymerase and T4 DNA polymerase, to produce a blunt ended 738 bp DNA fragment which was isolated and cloned into Asp I cut, klenow polymerase treated expression plasmid pQE30 (HIV). The resulting plasmid pBJ 1015 expresses HCV NS3 (246 amino acids) protease.

(ii) Construction of the plasmid pTS 56-9 with a stop codon after amino acid 183 (Figure 3)

The plasmid pTHB HCV NS3 was digested with Nco I, treated with klenow polymerase, then digested with Bst Y I; and the DNA fragment containing HCV sequence was isolated and cloned into Sma I and Bgl II digested pSP72. The resulting plasmid pTS 49-27 was then digested with Bgl II and Hpa I and ligated with a double stranded oligonucleotide:

GA TCA CCG GTC TAG ATCT

T GGC CAG ATC TAGA (SEQ ID NO 18) to produce pTS 56-9.

Thus, a stop codon was placed directly at the end of DNA encoding the

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protease catalytic domain of the NS3 protein. This enabled the HCV protease to be expressed independently from the helicase domain of the NS3 protein.

- 5 (iii) Construction of the plasmid pJB 1006 Fused with a peptide of positively charged amino acids at the carboxy terminus of NS3 183 (Figure 4).

10 The plasmid pTS 56-9 was digested with Sph I and Bgl II and the DNA fragment containing HCV sequence was isolated and cloned into a Sph I, Bgl II cut pSP72. The resulting plasmid pJB 1002 digested with Age I and HpaI and ligated to a double stranded oligonucleotide,

```

      CCG  GTC  CGG  AAG  AAA  AAG  AGA  CGC  TAG  C
          AG  GCC  TTC  TTT  TTC  TCT  GCG  ATC  G

```

- 15 (SEQ ID NO 19), to construct pJB 1006. This fused the hydrophilic, solubilizing motif onto the NS3 protease.

- 20 (iv) Construction of the plasmid pBJ 1022 expressing His-NS3(183)-HT in E.coli (Figure 5)

25 The plasmid pJB 1006 was digested with NgoM I and Nhe I and the 216 bp DNA fragment was isolated and cloned into Ngo M I, Nhe I cut pBJ 1015 to construct plasmid pBJ 1019. The plasmid pBJ 1019 was digested with Nar I and Pvu II, and treated with Klenow polymerase to fill in 5' ends of Nar I fragments. The expression plasmid pQE31 (Invitrogen) was digested with BamH I, blunt ended with Klenow polymerase. The 717 bp Nar I- Pvu II DNA fragment was isolated and ligated to the 2787 bp BamH I/Klenowed -Msc I (Bal I) fragment of the expression plasmid

30 pQE31 (Invitrogen). The recombinant plasmid, pBJ 1022, obtained after transformation into *E.coli* expresses His NS3(2-183)-HT which does not contain any HIV protease cleavage site sequence. The plasmid also contains a large deletion in the CAT (Chloramphenicol Acetyl Transferase) gene.

35

- (v) Construction of the plasmid pNB(-V)182-Δ4A HT (Figure 6)

- 22 -

The plasmid pMBM 48 was digested with Eag I and Xho I, treated with Klenow polymerase and the 320 bp DNA fragment was isolated and cloned into BamH I cut, blunt ended pSP 72 to construct the plasmid pJB1004. The 320 bp fragment encodes 7 amino acid from carboxy terminal of NS3(631), all of NS4A, and the amino terminal 46 amino acid of NS4B. The recombinant plasmid pJB1004 was digested with Eag I and Cel 2, blunt ended with Klenow polymerase. The 220 bp DNA fragment was isolated and cloned into the expression plasmid pQE30 which was digested with BamH I and blunt ended with Klenow polymerase prior to ligation. The resulting plasmid pJB 1011 was digested with NgoM I and Hind III and ligated to a double stranded oligonucleotide ,

CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAA TTC
GT TAA TAT GGA CTG TCC CTC CAA GAG ATG GTC CTT AAG

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC A
CTA CTC TAC CTT CTC ACG GCC TTC TTT TTC TCT GCG TTC GA
(SEQ ID NO 20)

to construct the plasmid pNB 4A HT. The plasmid pNB 4AHT was digested with Msl I and Xba I. The 1218 bp DNA fragment was isolated and cloned into Age I cut, klenow polymerase treated, Xba I cut vector DNA of pBJ 1019. The ligation results in a substitution of the 183rd amino acid residue valine by a glycine residue in NS3, and a deletion of amino terminal three amino acid residues of NS4A at the junction. The recombinant plasmid pNB182Δ4A HT comprising NS3(182aa)-G-NS4A(4-54 amino acid) does not contain NS3/NS4A cleavage site sequence at the junction and is not cleaved by the autocatalytic activity of NS3. Finally the plasmid pNB182Δ4A HT (SEQ ID NO 8) was digested with Stu I and Nhe I, the 803 bp DNA fragment was isolated and cloned into Stu I and Nhe I cut plasmid pBJ 1022. The resulting plasmid pNB(-V)182-Δ4A HT contains a deletion of the HIV sequence from the amino terminus end of the NS3 sequence and in the CAT gene (SEQ ID NO 23).

(vi) Construction of the plasmid pT5 His HIV-NS3 (Figure 7)

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The plasmid pTS56-9 was digested with Bgl II, and treated with Klenow polymerase to fill in 5' ends. The plasmid was then digested with NgoM I and the blunt ended Bgl II/NgoMI fragment containing the NS3 sequence was isolated and ligated to the SglI, Klenow treated
5 NgmMI cut and Sal I klenowed pBJ 1015. The resulting plasmid is designated pT5His HIV 183.

Example 4

10

Purification of HCV NS3 Protease having a Solubilizing Motif

Purification of His182HT (SEQ ID NO 4) and His (-V)182Δ4AHT (SEQ ID NO 8)

15

The recombinant plasmids pBJ1022 and pNB(-V)182Δ4A were used to transform separate cultures of *E. coli* strain M15 [pREP4] (Qiagen), which over-expresses the *lac* repressor, according to methods recommended by the manufacturer. M15 [pREP4] bacteria harboring
20 recombinant plasmids were grown overnight in broth containing 20g/L bactotrypton, 10g/L bacto-yeast extract, 5g/L NaCl (20-10-5 broth) and supplemented with 100μg/ml ampicillin and 25μg/ml kanamycin. Cultures were diluted down to O.D.600 of 0.1, then grown at 30°C to O.D.600 of 0.6 to 0.8, after which IPTG was added to a final concentration
25 of 1mM. At post-induction 2 to 3 hours, the cells were harvested by pelleting, and the cell pellets were washed with 100mM Tris, pH 7.5. Cell lysates were prepared as follows: to each ml equivalent of pelleted fermentation broth was added 50μl sonication buffer (50mM sodium phosphate, pH 7.8, 0.3M NaCl) with 1mg/ml lysozyme; cell suspension
30 was placed on ice for 30 min. Suspension was then brought to a final concentration of 0.2% Tween-20, 10mM dithiothreitol (DTT), and sonicated until cell breakage was complete. Insoluble material was pelleted at 12,000 x g in a microcentrifuge for 15 minutes, the soluble portion was removed to a separate tube and the soluble lysate was then
35 brought to a final concentration of 10% glycerol. Soluble lysates from cells expressing the plasmids produce strongly immunoreactive bands of the predicted molecular weight. Soluble lysates prepared for Ni²⁺

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column purification were prepared with 10mM β -mercaptoethanol (BME) instead of DTT. Lysates were stored at -80°C .

5 Purification using Ni^{2+} -Nitrosyl acetic acid (NTA) agarose (OIA GEN)

The proteins were then purified by placing the extracted lysate on an NTA agarose column. NTA agarose column chromatography was used because the histidine tag which was fused to the N-terminus of the
10 proteases readily binds to the nickel column. This produces a powerful affinity chromatographic technique for rapidly purifying the soluble protease. The column chromatography was performed in a batch mode. The Ni^{2+} NTA resin (3ml) was washed twice with 50 ml of Buffer A (50mM sodium phosphate pH 7.8 containing 10% glycerol, 0.2% Tween-
15 20, 10mM BME). The lysate obtained from a 250 ml fermentation (12.5 ml) was incubated with the resin for one hour at 4°C . The flow through was collected by centrifugation. The resin was packed into a 1.0×4 cm column and washed with buffer A until the baseline was reached. The bound protein was then eluted with a 20 ml gradient of imidazole (0-
20 0.5M) in buffer A. Eluted fractions were evaluated by SDS-PAGE and western blot analysis using a rabbit polyclonal antibody to His-HIV 183.

Purification using POROS metal-chelate affinity column

25 In an alternative method to purify the proteins the lysate containing the proteins were applied to a POROS metal-chelate affinity column. Perfusion chromatography was performed on a POROS MC metal chelate column ($4.6 \times 50\text{mm}$, 1.7 ml) precharged with Ni^{2+} . The sample was applied at 10 ml/min and the column was washed with buffer A.
30 The column was step eluted with ten column volumes of buffer A containing 25 mM imidazole. The column was further eluted with a 25 column volume gradient of 25-250 mM imidazole in buffer A. All eluted fractions were evaluated by SDS-PAGE and western blot analysis using rabbit polyclonal antibody.

35

Example 5

Peptide Synthesis of the 5A/5B and 4B/5A Substrates

- 25 -

The peptides 5A/5B and 4B/5A substrates (SEQ ID NOs 16, 18, 19, 20 and 21) were synthesized using Fmoc chemistry on an ABI model 431A peptide synthesizer. The manufacture recommended FastMoc™
5 activation strategy (HBTU/HOBt) was used for the synthesis of 4A activator peptide. A more powerful activator, HATU with or without the additive HOAt were employed to assemble 5A/5B substrate peptides on a preloaded Wang resin. The peptides were cleaved off the resin and deprotected by standard TFA cleavage protocol. The peptides were
10 purified on reverse phase HPLC and confirmed by mass spectrometric analysis.

Example 6

15 HPLC-assay using a synthetic 5A/5B peptide substrate

To test the proteolytic activity of the HCV NS3 protease the DTEDVVCC SMSYTWGK (SEQ ID NO 16) and soluble HCV NS3 (SEQ ID NO 27) were placed together in an assay buffer. The assay buffer was
20 50mM sodium phosphate pH 7.8, containing 15% glycerol, 10mM DTT, 0.2% Tween20 and 200 mM NaCl). The protease activity of SEQ ID NO 27 cleaved the substrate into two byproduct peptides, namely 5A and 5B. The substrate and two byproduct peptides were separated on a reversed-phase HPLC column. (Dynamax, 4.6 x 250 mm) with a pore size of 300Å
25 and a particle size of 5µm. The column was equilibrated with 0.1%TFA (Solvent A) at a flow rate of 1 ml per minute. The substrate and the product peptide standards were applied to the column equilibrated in A. Elution was performed with a acetonitrile gradient (Solvent B=100% acetonitrile in A). Two gradients were used for elution (5% to 70%B in
30 50 minutes followed by 70% to 100%B in 10 minutes).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: Schering Corp.

(ii) TITLE OF INVENTION: Synthetic Inhibitors of Hepatitis C Virus
NS3 Protease

10 (iii) NUMBER OF SEQUENCES: 30

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering Corp.
(B) STREET: 2000 Galloping Hill Road
15 (C) CITY: Kenilworth
(D) STATE: New Jersey
(E) COUNTRY: USA
(F) ZIP: 07033-0530

20 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 7.1
(D) SOFTWARE: Microsoft Word 5.1a

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

30

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/644,544
(B) FILING DATE: 10 May 1996

35 (viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Dulak, Norman C.
(B) REGISTRATION NUMBER: 31,608

- 27 -

(C) REFERENCE/DOCKET NUMBER: JB0595

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 908-298-5061

5 (B) TELEFAX: 908-298-5388

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

20 Glu Asp Val Val Cys Cys Acp Acp Cys Val Val Ile Val Gly Arg
5 10 15
Ile Val Leu Ser Gly Lys
20

25 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

30 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

35 (ix) FEATURE:

(A) NAME/KEY:

- 28 -

Glu Asp Val Val Cys Cys Acp Cys Val Val Ile Val Gly Arg Ile
 5 10 15
 Val Leu Ser Gly Lys Lys
 20

5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

10

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

15

(ix) FEATURE:

(A) NAME/KEY:

Glu Asp Val Val Cys Cys Acp Lys Lys Gly Ser Leu Val Ile Arg
 5 10 15
 Gly-Val-Ile-Val-Val-Cys
 20

20

(2) INFORMATION FOR SEQ ID NO:4:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

30

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

35

(A) NAME/KEY:

(B) OTHER INFORMATION: Xaa is lysine having a peptide bond
 between its ϵ -amino group and the carboxyl group of lysine at position 8.

- 29 -

The carboxyl group of the Xaa forms a peptide bond with the α -amino group of another lysine (not shown);

```

      Glu Asp Val Val Cys Cys Xaa Lys Gly Ser Leu Val Ile Arg Gly
5      5      10      15
      Val Ile Val Val Cys
      20

```

(2) INFORMATION FOR SEQ ID NO:5:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

15

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

20

(A) NAME/KEY:

(B) OTHER INFORMATION: Amino acid residues at positions 9-21 are preferably D-amino acid residues;

```

      Glu Asp Val Val Cys Cys Acp Acp Lys Gly Ser Leu Val Ile Arg
      5      10      15
25     Gly Val Ile Val Val Cys
      20

```

(2) INFORMATION FOR SEQ ID NO:6:

30

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

35

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- 31 -

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

5

(ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION:

10 Xaa is a lysine wherein the ϵ amino group of which forms a peptide bond with the carboxyl group of the cysteine residue at position 8 and the carboxyl group of the lysine residue forms a peptide bond with an α amino group of another lysine residue (not shown), preferably the amino acid residues at positions 8 - 20 are D- amino acid residues.

15 Glu Asp Val Val Cys Cys Xaa Cys Val Val Ile Val Gly Arg Ile
 5 10 15
 Val Leu Ser Gly Lys
 20

20 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

25 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

30 (ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues at positions 1- 13 are preferably D-amino acid residues and lysine at position 14 is preferably an L-amino acid residue;

35

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Lys
 5 10

- 32 -

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

10

(ix) FEATURE:

(A) NAME/KEY:

(B) OTHER INFORMATION: Amino acid residues at positions 1 -
11 are preferably D-amino acids;

15

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Lys
5 10

INFORMATION FOR SEQ ID NO:11:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
25 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

30

(A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues are
preferably D-amino acid residues.

35

Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly
5 10

INFORMATION FOR SEQ ID NO:12:

- 33 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

5 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

10 (A) NAME/KEY:

(B) OTHER INFORMATION: The amino acid residues are preferably D-amino acids and the serine residue at position 1 is preferably acetylated;

15 Ser-Leu-Val-Ile-Arg-Gly-Val-Ile-Val

5

INFORMATION FOR SEQ ID NO:13:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

25

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

30 (B) OTHER INFORMATION: The amino acid residues are preferably D-amino acid residues and the lysine residue at position 1 is preferably acetylated.

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys

35

5

10

INFORMATION FOR SEQ ID NO:14:

- 34 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

5 (D) TOPOLOGY:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

10 (A) NAME/KEY:

(B) OTHER INFORMATION: Xaa is biotin and the amino acid residues at positions 2 - 14 are preferably D-amino acids;

Xaa Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Lys

15

5

10

Lys

INFORMATION FOR SEQ ID NO:15:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

25

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY:

30 (B) OTHER INFORMATION: Xaa is a lysine residue in which the ϵ amino group of the lysine forms a peptide bond with a biotin and amino acid residues at positions 1 - 13 are preferably D-amino acid residues.

Lys Gly Ser Leu Val Ile Arg Gly Val Ile Val Val Cys Xaa Lys

35

5

10

15

(2) INFORMATION FOR SEQ ID NO:16:

- 35 -

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 549 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

5 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

10 (A) NAME/KEY: HCV NS3 Protease

GCG CCC ATC ACG GCG TAC GCC CAG CAG ACG AGA GGC CTC CTA GGG 45
 Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly
 1 5 10 15

15

TGT ATA ATC ACC AGC CTG ACT GGC CGG GAC AAA AAC CAA GTG GAG 90
 Cys Ile Ile Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu
 20 25 30

20

GGT GAG GTC CAG ATC GTG TCA ACT GCT ACC CAA ACC TTC CTG GCA 135
 Gly Glu Val Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala
 35 40 45

25

ACG TGC ATC AAT GGG GTA TGC TGG ACT GTC TAC CAC GGG GCC GGA 180
 Thr Cys Ile Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly
 50 55 60

30

ACG AGG ACC ATC GCA TCA CCC AAG GGT CCT GTC ATC CAG ATG TAT 225
 Thr Arg Thr Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr
 65 70 75

35

ACC AAT GTG GAC CAA GAC CTT GTG GGC TGG CCC GCT CCT CAA GGT 270
 Thr Asn Val Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly
 80 85 90

TCC CGC TCA TTG ACA CCC TGC ACC TGC GGC TCC TCG GAC CTT TAC 315
 Ser Arg Ser Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr

- 36 -

| | | | |
|----|---|-----|-----|
| | 95 | 100 | 105 |
| | CTG GTT ACG AGG CAC GCC GAC GTC ATT CCC GTG CGC CGG CGA GGT 360 | | |
| | Leu Val Thr Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly | | |
| 5 | 110 | 115 | 120 |
| | GAT AGC AGG GGT AGC CTG CTT TCG CCC CGG CCC ATT TCC TAC CTA 405 | | |
| | Asp Ser Arg Gly Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu | | |
| | 125 | 130 | 135 |
| 10 | AAA GGC TCC TCG GGG GGT CCG CTG TTG TGC CCC GCG GGA CAC GCC 450 | | |
| | Lys Gly Ser Ser Gly Gly Pro Leu Leu Cys Pro Ala Gly His Ala | | |
| | 140 | 145 | 150 |
| 15 | GTG GGC CTA TTC AGG GCC GCG GTG TGC ACC CGT GGA GTG ACC AAG 495 | | |
| | Val Gly Leu Phe Arg Ala Ala Val Cys Thr Arg Gly Val Thr Lys | | |
| | 155 | 160 | 165 |
| | GCG GTG GAC TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC ATG AGA 540 | | |
| 20 | Ala Val Asp Phe Ile Pro Val Glu Asn Leu Glu Thr Thr Met Arg | | |
| | 170 | 175 | 180 |
| | TCC CCG GTG | | |
| | Ser Pro Val | | |

25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- 30
- (A) LENGTH: 162 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(ix) FEATURE:

- (A) NAME/KEY: NS4A

- 37 -

AGC ACC TGG GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG 45
 Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
 1 5 10 15

5 TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC 90
 Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val
 20 25 30

TTG TCC GGG AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC 135
 10 Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr
 35 40 45

CAG GAG TTC GAT GAG ATG GAA GAG TGC 162
 Gln Glu Phe Asp Glu Met Glu Glu Cys
 15 50

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 15 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: double

25 (ii) MOLECULE TYPE: cDNA

GA TCA CCG GTC TAG ATCT
 T GGC CAG ATC TAGA

30 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- 38 -

(ix) FEATURE:

(A) NAME/KEY:

5 CCG GTC CGG AAG AAA AAG AGA CGC TAG C
AG GCC TTC TTT TTC TCT GCG ATC G

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 79 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY:

20 CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAA TTC
GT TAA TAT GGA CTG TCC CTC CAA GAG ATG GTC CTT AAG

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC A
CTA CTC TAC CTT CTC ACG GCC TTC TTT TTC TCT GCG TTC GA

25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

30 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

35

(ix) FEATURE:

(A) NAME/KEY: NS4A Active Mutant

- 39 -

Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly Lys

5

10

(2) INFORMATION FOR SEQ ID NO:22:

5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

15 (A) NAME/KEY: Soluble 5A/5B Substrate

Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr

5

10

15

Gly Lys

20

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 810 base pairs

25 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(ix) FEATURE:

(A) NAME/KEY: pNB182Δ4AHT

35 ATG AGA GGA TCG CAT CAC CAT CAC CAT CAC ACG GAT CCG CCC ATC 45

Met Arg Gly Ser His His His His His His Thr Asp Pro Pro Ile

1

5

10

15

- 40 -

| | | |
|----|---|-----|
| | ACG GCG TAC GCC CAG CAG ACG AGA GGC CTC CTA GGG TGT ATA ATC | 90 |
| | Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile | |
| | 20 25 30 | |
| 5 | ACC AGC CTG ACT GGC CGG GAC AAA AAC CAA GTG GAG GGT GAG GTC | 135 |
| | Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val | |
| | 35 40 45 | |
| | CAG ATC GTG TCA ACT GCT ACC CAA ACC TTC CTG GCA ACG TGC ATC | 180 |
| 10 | Gln Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr Cys Ile | |
| | 50 55 60 | |
| | AAT GGG GTA TGC TGG ACT GTC TAC CAC GGG GCC GGA ACG AGG ACC | 225 |
| 15 | Asn Gly Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr | |
| | 65 70 75 | |
| | ATC GCA TCA CCC AAG GGT CCT GTC ATC CAG ATG TAT ACC AAT GTG | 270 |
| | Ile Ala Ser Pro Lys Gly Pro Val Ile Gln Met Tyr Thr Asn Val | |
| 20 | 80 85 90 | |
| | GAC CAA GAC CTT GTG GGC TGG CCC GCT CCT CAA GGT TCC CGC TCA | 315 |
| | Asp Gln Asp Leu Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser | |
| 25 | 95 100 105 | |
| | TTG ACA CCC TGC ACC TGC GGC TCC TCG GAC CTT TAC CTG GTT ACG | 360 |
| | Leu Thr Pro Cys Thr Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr | |
| 30 | 110 115 120 | |
| | AGG CAC GCC GAC GTC ATT CCC GTG CGC CGG CGA GGT GAT AGC AGG | 405 |
| | Arg His Ala Asp Val Ile Pro Val Arg Arg Arg Gly Asp Ser Arg | |
| 35 | 125 130 135 | |

- 41 -

GGT AGC CTG CTT TCG CCC CGG CCC ATT TCC TAC CTA AAA GGC TCC 450
 Gly Ser Leu Leu Ser Pro Arg Pro Ile Ser Tyr Leu Lys Gly Ser
 140 145 150

5 TCG GGG GGT CCG CTG TTG TGC CCC GCG GGA CAC GCC GTG GGC CTA 495
 Ser Gly Gly Pro Leu Leu Cys Pro Ala Gly His Ala Val Gly Leu
 155 160 165

TTC AGG GCC GCG GTG TGC ACC CGT GGA GTG ACC AAG GCG GTG GAC 540
 10 Phe Arg Ala Ala Val Cys Thr Arg Gly Val Thr Lys Ala Val Asp
 170 175 180

TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC ATG AGA TCC CCG GGG 585
 Phe Ile Pro Val Glu Asn Leu Glu Thr Thr Met Arg Ser Pro Gly
 15 185 190 195

GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG TAT TGC CTG 630
 Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala Tyr Cys Leu
 200 205 210

20 TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC TTG TCC GGG 720
 Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val Leu Ser Gly
 215 220 225

AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC CAG GAG TTC 765
 25 Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr Gln Glu Phe
 230 235 240

GAT GAG ATG GAA GAG TGC CGG AAG AAA AAG AGA CGC AAG CTT AAT 810
 30 Asp Glu Met Glu Glu Cys Arg Lys Lys Lys Arg Arg Lys Leu Asn
 245 250 255

(2) INFORMATION FOR SEQ ID NO:24:

- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 162 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

- 42 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5 (ix) FEATURE:

(A) NAME/KEY: Native NS4A

TCA ACA TGG GTG CTC GTT GGC GGC GTC CTG GCT GCT CTG GCC GCG 45
 Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala Ala Leu Ala Ala
 10 1 5 10 15

 TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC AGG ATT GTC 90
 Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly Arg Ile Val
 20 25 30
 15
 TTG TCC GGG AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT CTC TAC 135
 Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val Leu Tyr
 35 40 45
 20 CAG GAG TTC GAT GAG ATG GAA GAG TGC
 Gln Glu Phe Asp Glu Met Glu Glu Cys
 50

2) INFORMATION FOR SEQ ID NO:25:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

30

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

35

(A) NAME/KEY: Native 5A/5B Substrate

- 43 -

Asp Thr Glu Asp Val Val Cys Cys Ser Met Ser Tyr Thr Trp Thr
5 10 15
Gly

5 2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

10 (C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

15 (ix) FEATURE:

(A) NAME/KEY: NS3/NS4A Cleavage site

Cys Met Ser Ala Asp Leu Glu Val Val Thr Ser Thr Trp Val Leu
 5 10 15
 20 Val Gly Gly Val Leu
 20

2) INFORMATION FOR SEQ ID NO:27:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY: NS4A/4B Cleavage Site

35 Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
5 10 15
Tyr Ile Glu Gln Gly
20

- 44 -

2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

- (A) NAME/KEY: 4B/5A

15 Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys Ser Gly Ser Trp Leu
5 10 15
Arg Asp Ile Trp Asp
20

20 2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
25 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

30 (ix) FEATURE:

- (A) NAME/KEY:

Glu-Asp-Val-Val-Cys-Cys-Acp-Lys
5

35

2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- 45 -

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY:

10

Cys-Val-Val-Ile-Val-Gly-Arg-Ile-Val-Leu-Ser-Gly-Lys

5

10

WE CLAIM:

1. A bivalent inhibitor of an hepatitis C NS3 protease comprised of a
5 first peptide linked to a second peptide, said first peptide being a
subsequence, a mutated subsequence or a mutated full-length sequence
of a substrate of the hepatitis C NS3 protease and said second peptide
being a subsequence of a hepatitis C NS4A polypeptide.
- 10 2. The bivalent inhibitor of claim 1 selected from the group consisting
of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,
SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.
3. An inhibitor of an HCV protease comprised of a peptide, said peptide
15 being a subsequence, a mutated subsequence or a mutated full-length
sequence of a substrate of the HCV NS3 protease.
4. An inhibitor of claim 3 selected from the group consisting of SEQ ID
NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13,
20 SEQ ID NO: 14 and SEQ ID NO: 15.
5. An inhibitor of an HCV NS3 protease comprised of a peptide, said
peptide being a subsequence, a mutated subsequence or a mutated full-
length sequence of an HCV NS4A polypeptide.
25
6. The use of an inhibitor of an HCV NS3 protease for the manufacture
of a medicament for treating hepatitis C, wherein the inhibitor is
comprised of a first peptide linked to a second peptide, said first peptide
being a subsequence, mutated subsequence or a mutated full-length
30 sequence of a substrate of the hepatitis C NS3 protease and said second
peptide being a subsequence, a mutated subsequence or a mutated full-
length sequence of a hepatitis C NS4A polypeptide.
7. The use of claim 6 wherein the inhibitor is selected from the group
35 consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4,
SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and SEQ ID NO: 8.

8. The use of an inhibitor of an HCV NS3 protease for the manufacture of a medicament for treating hepatitis C, wherein the inhibitor is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV
5 NS3 protease.

9. The use of claim 8 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

10

10. The use of an inhibitor of an HCV NS3 protease for the manufacture of a medicament for treating hepatitis C, wherein the inhibitor is comprised of a peptide, said peptide being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV
15 NS4A polypeptide.

11. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being an inhibitor of an HCV NS3 protease, said inhibitor being comprised of a
20 first peptide linked to a second peptide, said first peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the hepatitis C NS3 protease and said second peptide being a subsequence, a mutated subsequence or a mutated full-length sequence of a hepatitis C NS4A polypeptide, and a pharmaceutical
25 carrier.

12. The pharmaceutical composition of claim 11 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and
30 SEQ ID NO: 8.

13. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical
35 carrier, said inhibitor being a subsequence, a mutated subsequence or a mutated full-length sequence of a substrate of the HCV NS3 protease.

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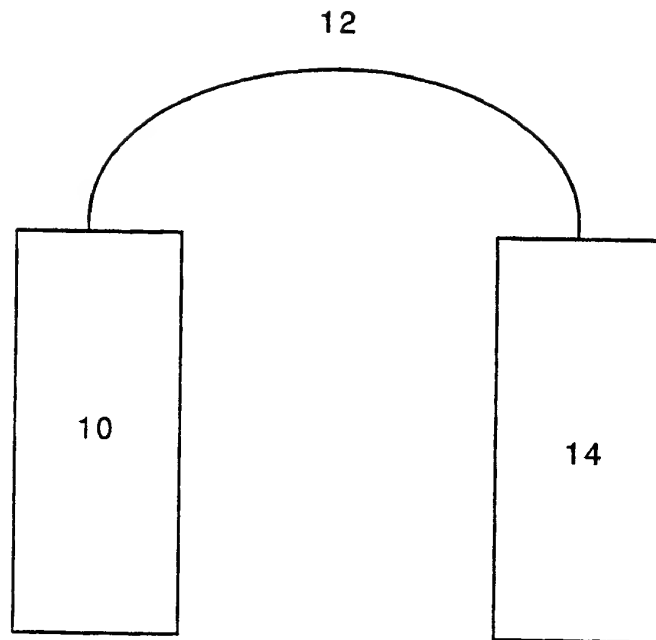
14. The pharmaceutical composition of claim 13 wherein the inhibitor is selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 15.

5

15. A pharmaceutical composition for treating an individual infected with hepatitis C virus, said pharmaceutical composition being comprised of an inhibitor of an HCV NS3 protease and a pharmaceutical carrier, wherein said inhibitor is comprised of a peptide, said peptide
10 being a subsequence, a mutated subsequence or a mutated full-length subsequence of an HCV NS4A polypeptide.

15

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**Fig. 1****SUBSTITUTE SHEET (RULE 26)**

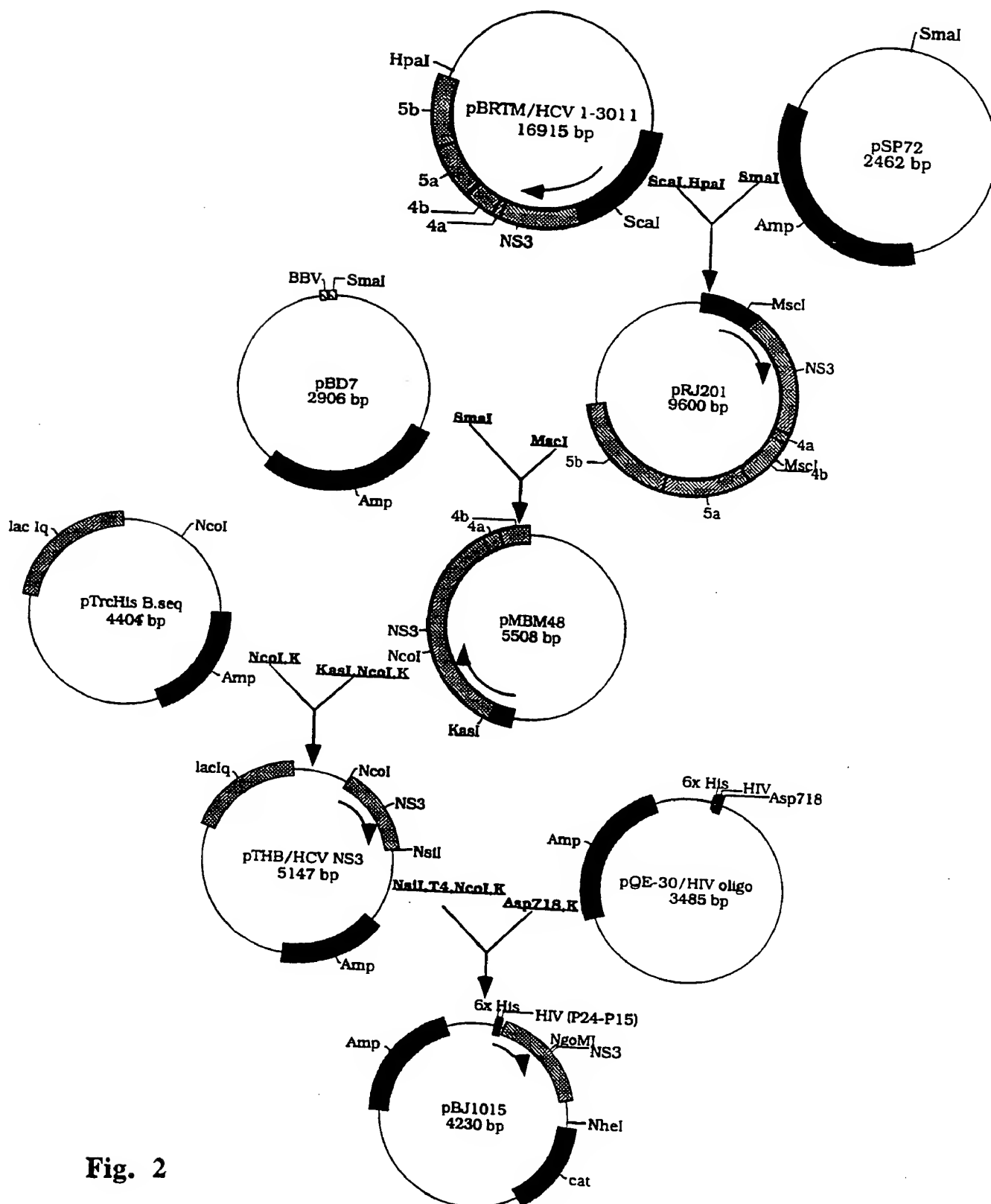


Fig. 2

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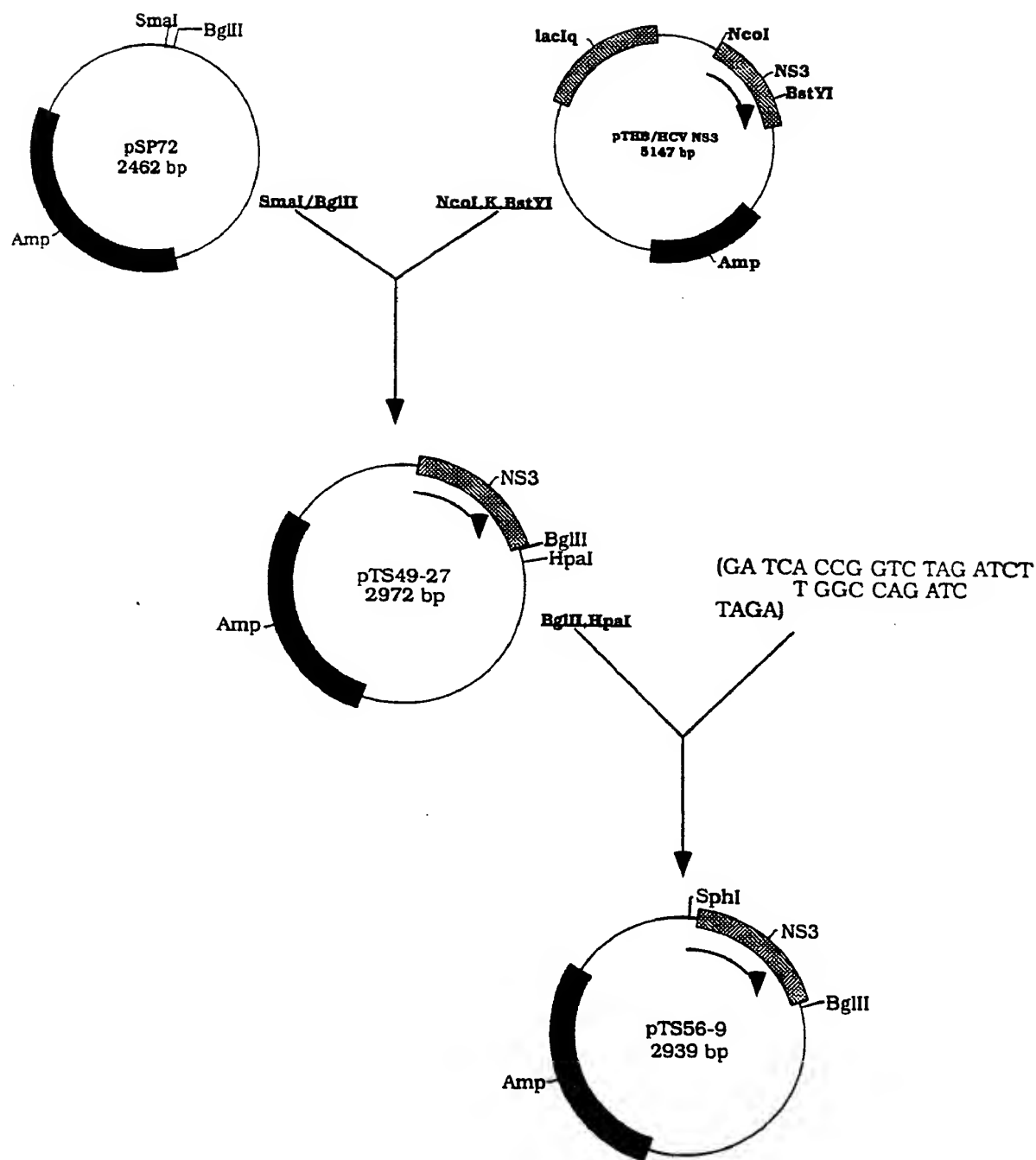


Fig. 3

SUBSTITUTE SHEET (RULE 26)

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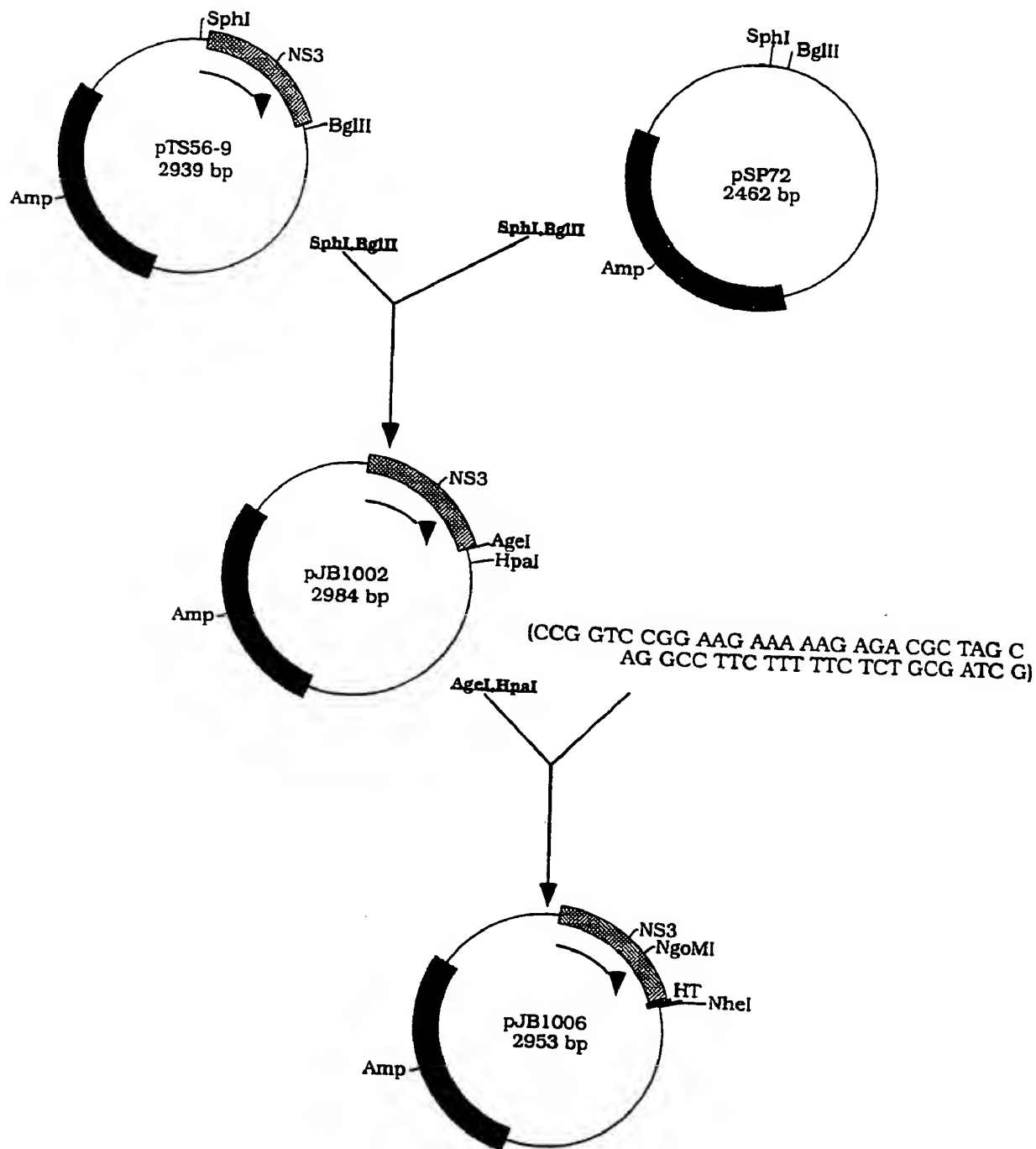


Fig. 4

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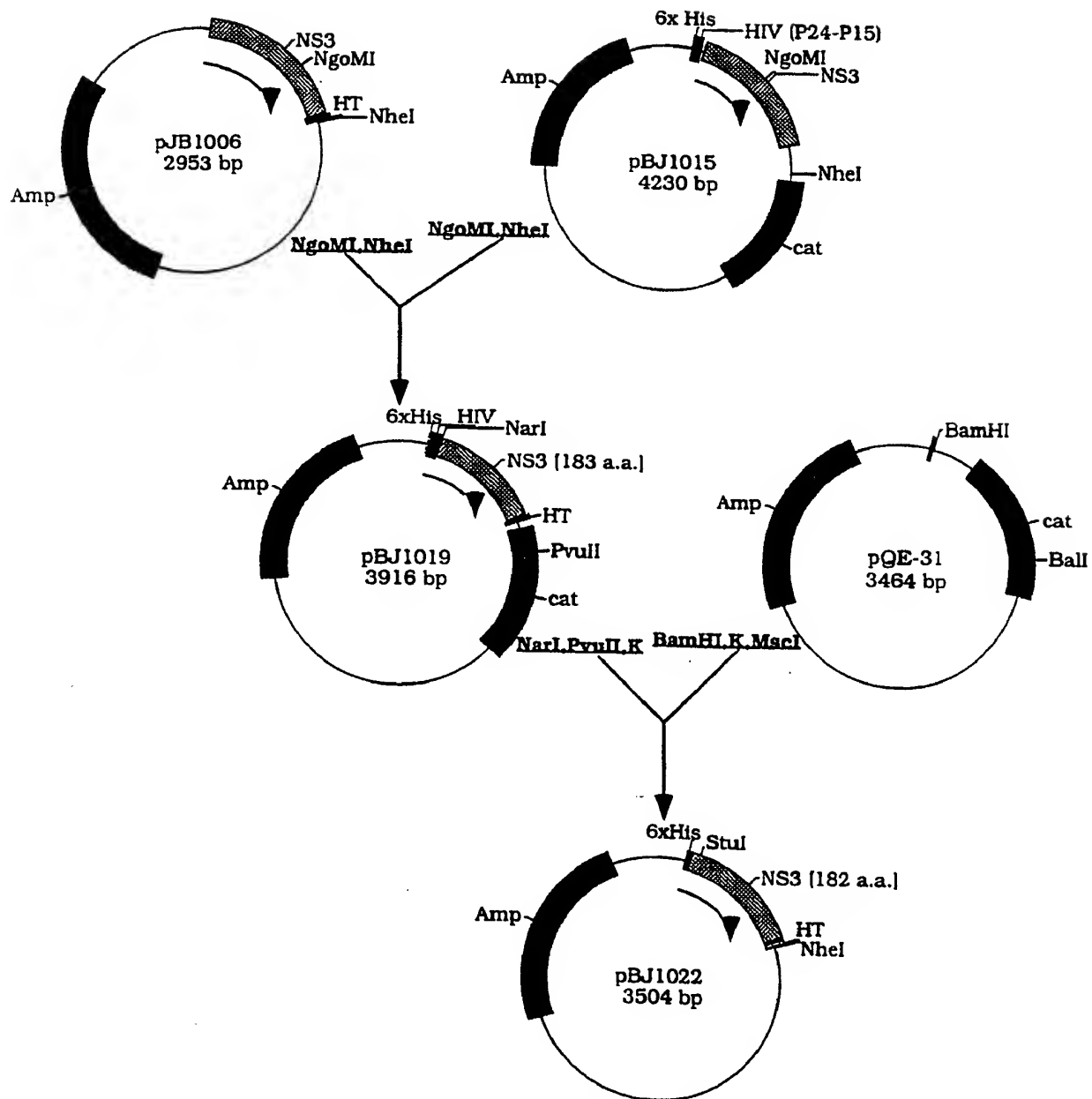


Fig. 5

SUBSTITUTE SHEET (RULE 26)

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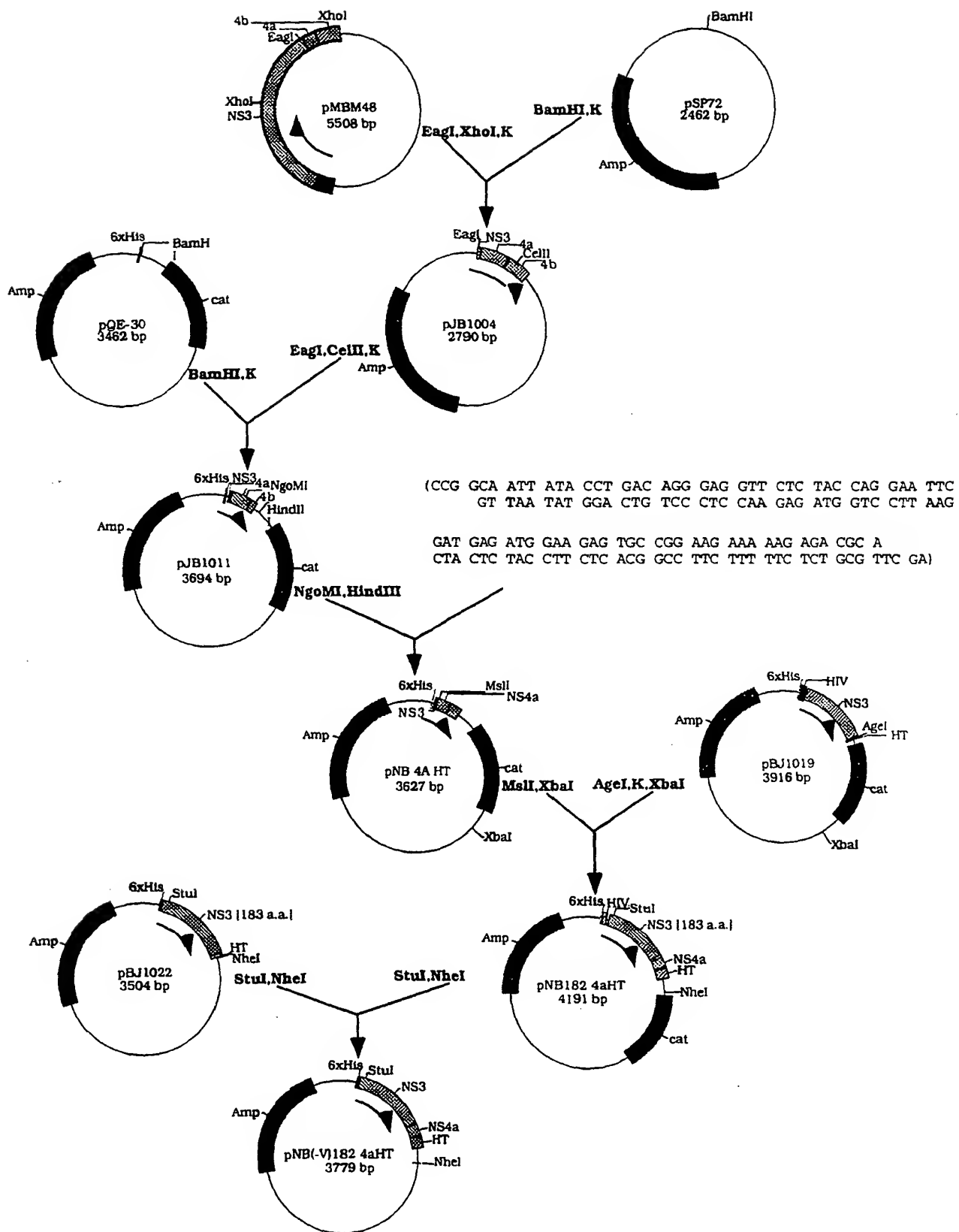


Fig. 6
SUBSTITUTE SHEET (RULE 26)

7/7

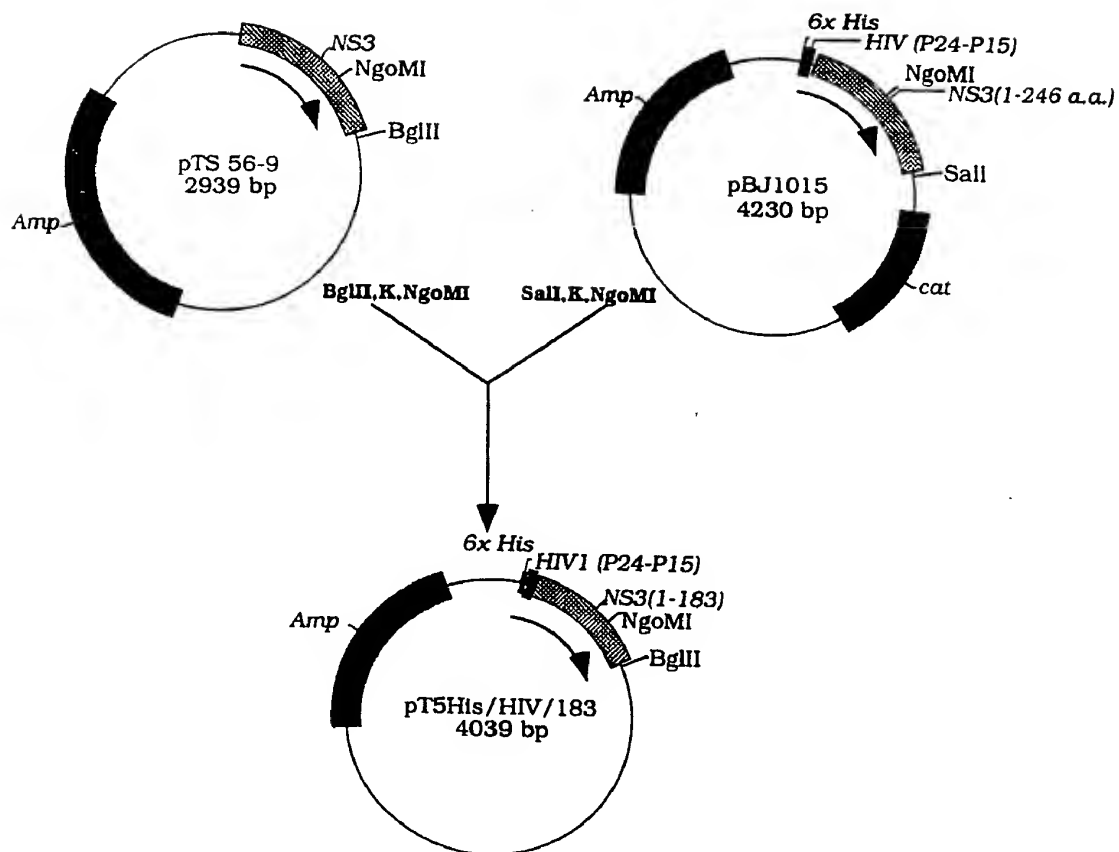


Fig. 7

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/07632

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/18 C07K19/00 A61K39/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| A | WO 95 22985 A (ISTITUTO DI RICERCHE DI BIOLOG ;FRANCESCO RAFFAELE DE (IT); FAILLA) 31 August 1995 see page 3, last paragraph - page 4, paragraph 3; example 4 --- | 1-15 |
| A | HIROAKI OKAMOTO ET AL.: "The 5'-terminal sequence of the Hepatitis C Virus genome " THE JAPANESE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 60, no. 1, January 1990, pages 167-177, XP002042711 see the whole document --- -/- | 1-15 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

7 October 1997

Date of mailing of the international search report

28.10.97

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/07632

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| P,X | WO 96 36702 A (SCHERING CORPORATION) 21 November 1996 see page 3, line 15 - line 19 see page 6, line 12 - line 20 see page 13, line 27 - page 14, line 10; example 3 --- | 1,3,6,8, 11,13 |
| P,X | WO 96 35806 A (SCHERING CORPORATION) 14 November 1996 see page 6, line 35 - page 7, line 1; example 5 --- | 3,8,13 |
| P,X | WO 96 35717 A (SCHERING CORPORATION) 14 November 1996 see page 4, line 10 - line 37 see page 13, line 15 - line 37; example 3 ----- | 3,8,13 |

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. Application No
PCT/US 97/07632


| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|--|----------------------------------|
| WO 9522985 A | 31-08-95 | AU 1822395 A CA 2182521 A EP 0746333 A | 11-09-95 31-08-95 11-12-96 |
| ----- | ----- | ----- | ----- |
| WO 9636702 A | 21-11-96 | AU 5729196 A | 29-11-96 |
| ----- | ----- | ----- | ----- |
| WO 9635806 A | 14-11-96 | AU 5729096 A | 29-11-96 |
| ----- | ----- | ----- | ----- |
| WO 9635717 A | 14-11-96 | AU 5729296 A | 29-11-96 |
| ----- | ----- | ----- | ----- |

Form PCT/ISA/210 (patent family annex) (July 1992)

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | | |
|--|--|---|--|
| Applicant's or agent's file reference KMN/FP5780044 | | FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) | |
| International application No. PCT/GB99/01824 | International filing date (day/month/year) 09/06/1999 | Priority date (day/month/year) 10/06/1998 | |
| International Patent Classification (IPC) or national classification and IPC C07K5/06 | | | |
| Applicant ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE..et al | | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p> | | | |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none">I <input checked="" type="checkbox"/> Basis of the reportII <input type="checkbox"/> PriorityIII <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicabilityIV <input checked="" type="checkbox"/> Lack of unity of inventionV <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statementVI <input type="checkbox"/> Certain documents citedVII <input checked="" type="checkbox"/> Certain defects in the international applicationVIII <input type="checkbox"/> Certain observations on the international application | | | |
| Date of submission of the demand 15/12/1999 | | Date of completion of this report 04.09.2000 | |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | | Authorized officer Döpfer, K-P Telephone No. +49 89 2399 8547 | |



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/01824

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-92 as originally filed

Claims, No.:

1-30 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
☒ claims Nos. 29 (partially).

because:

- ☒ the said international application, or the said claims Nos. 29 (partially) relate to the following subject matter which does not require an international preliminary examination (*specify*):



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/01824

see s parat sheet

- ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

- ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

- ☐ no international search report has been established for the said claims Nos. .

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB99/01824

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

| | | | |
|-------------------------------|------|--------|--|
| Novelty (N) | Yes: | Claims | 1-30 |
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-19 (all partially), 20-24, 25-29 (all partially), 30 |
| | No: | Claims | 1-19, 25-29 (all partially) |
| Industrial applicability (IA) | Yes: | Claims | 1-28, 30 |
| | No: | Claims | |

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet



Re Item I

Basis of the report

1. The sequence listing with the separate pages 1-10 are considered part of the application documents as originally filed.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claim 29 relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
Nevertheless, an International preliminary examination on novelty and inventive step of the subject-matter of the above mentioned claims is being carried out with respect to the alleged effects underlying said methods see Item V of this report).

Re Item IV

Lack of unity of invention

1. Due to the lack of inventive step of peptide analogues characterised by the sole substitution of the type of fluorinated side chain (see Item V of this report) no special technical feature according to Rule 13(2) PCT is anymore present which could serve as linking feature in order to establish unity of the invention as stipulated by Rule 13(1) PCT. Each modification of the peptide, either at the termini (moiety X or X' of the general formula) or in the sequence (including side chains), would represent a separate invention.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98 22496 A (HOFFMANN LA ROCHE) 28 May 1998 (1998-05-28)
D2: WO 97 36587 A (MERCK & CO INC ;HEIMBROOK DAVID C (US); OLIFF
ALLEN I (US); STIRDI) 9 October 1997 (1997-10-09)
D3: WO 97 43310 A (SCHERING CORP) 20 November 1997 (1997-11-20)

2. The present application relates to fluorine containing oligopeptides of the general formulae Y-B-A-X (I) or Y-B-A'-X' (II) which can act as inhibitors of the HCV NS3 protease, to uses of such compounds and to their preparation.

2.1 Novelty (Article 33(2) PCT)

D1 discloses peptide aldehydes bearing CF_3 and CHF_2 groups in the side chain of the amino acid sequence which exhibit inhibitory activity towards HCV proteases. The substitution pattern in the primary sequence of the amino acids is distinct from the combination disclosed in the present application as presented in the definitions for the general formulae (I) and (II).

The compounds of formula (I) must comprise $\text{CH}_2\text{-CF}_2\text{H}$ as fluorine containing side chain. This particular embodiment is not disclosed in D1.

The compounds of formula (II) are novel because only -OH and $\text{-NHSO}_2\text{R}_{25}$ are allowed at the position X'.

Therefore, novelty can be acknowledged for all claims in view of the disclosure of D1.

D2 relates to antineoplastically active peptides acting as inhibitors of the farnesyl protein transferase. These compounds comprise glycine at position B' of general formula (II) which is not within the definition of B for the present application.

Furthermore, the structural requirements concerning Y' are not met either. Thus, the present application is considered novel over D2 for the subject-matter of all claims.

D3 pertains to chimeric peptides comprising (mutated) subsequences or a full-

length sequence of a substrate of the HCV NS3 protease and a subsequence of a HCV NS4A polypeptide. These peptides are inhibitors of the HCV NS3 protease. The structure of these peptides is remote from those of the peptide derivatives of the present application. Hence, the teaching of this document is considered as representing state of the art which is not pertinent for the assessment of novelty.

The subject-matter concerning the fluorinated peptide ketoacid derivatives (which is the only sufficiently supported one) and the method of synthesis (Claim 30) is considered novel in view of the prior art (see also point 2.1 of Item V and Item IV of this report).

2.2 Inventive step (Article 33(3) PCT)

D1 is considered representing the closest prior art. The problem underlying the present application is regarded as to provide further peptide derivatives which exhibit inhibitory activity towards NS3A protease of HCV in order to obtain a therapeutic agent against HCV infection.

The solution are fluorinated peptide derivatives, in particular ketoacids of formulae (I) and (II) with $X = -\text{COOH}$ or $-\text{CONR}^9\text{R}^{10}$. D1 discloses different "C-terminal" modifications of fluorinated peptide inhibitors of the HCV NS3A protease like amino aldehydes ($X = \text{H}$), or boronic acid analogues. Neither the teaching of D1 alone nor the combination with one of the other prior art documents would have lead the skilled person to the claimed solution of the technical problem posed. The inhibitory activity as demonstrated in the tables of the present application could not expected from the prior art. Accordingly, inventive step can be acknowledged for the subject-matter relating to the keto acid analogues (at least claims 20-24; partially all claims 1-19, 25-29). Furthermore, the particular synthetic sequence for obtaining the active compounds is not obvious in view of the prior art either (Claim 30).

The subject-matter which relates to the sole introduction of the particular fluorinated side chain $-\text{CH}-\text{CF}_2\text{H}$ appears not to involve an inventive step because



such a substitution is a matter of normal experimental design for the skilled person, and, furthermore the present application does not disclose any surprising effect or particular advantage over the compounds of D1 (i.e. inventive step is lacking partially for Claims 1-19, 25-29).

D2, which discloses structurally similar compounds, pertains to a different technical field (HCV protease inhibition vs. inhibition of farnesyl protein transferase in order to treat cancer). The skilled person would not take this document into consideration to modify compounds of D1 to solve the technical problem as defined.

2.4 Industrial applicability (Article 33(4) PCT)

The subject-matter of present claims 1-28 and 30 meets the requirements of industrial applicability as stipulated in Article 33(4) PCT. Concerning present claim 29 see Item III of this written opinion.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D3 are not mentioned in the description, nor are these documents identified therein.
2. The temperature (page 49, example 4 i)) should read 50°C (instead of 50% C) (Rule 10.1(b) PCT). The unit mL for volumes should read ml (Rule 10.1(a) PCT).

INTERNATIONAL SEARCH REPORT

International Application No

PC1, GB 99/01824

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/06 C07K5/08 C07K7/06 C07C237/22 C07C311/45
C07D209/26 C07D209/32 C07D307/94 C07D333/24 C07D407/12
C07D409/06 C07D409/12 A61K38/05 A61K38/06 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | WO 98 22496 A (HOFFMANN LA ROCHE) 28 May 1998 (1998-05-28) page 2, line 10 - line 14; claims; examples 3,8,17-19,47-58,61,62 page 15, line 14 -page 18, line 12 --- | 1-29 |
| X | WO 97 36587 A (MERCK & CO INC ;HEIMBROOK DAVID C (US); OLIFF ALLEN I (US); STIRDI) 9 October 1997 (1997-10-09) page 298, line 5 - line 6; claims; examples page 299, line 1 - line 2 --- | 1,26 |
| A | WO 97 43310 A (SCHERING CORP) 20 November 1997 (1997-11-20) claims; examples ----- | 1-29 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

15 September 1999

Date of mailing of the international search report

22/09/1999

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Fuhr, C

INTERNATIONAL SEARCH REPORT

International Application No.

PC1/GB 99/01824

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/195 A61K31/335 A61K31/38 A61K31/405

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| | | |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

15 September 1999

Date of mailing of the international search report

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Fuhr, C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/ 01824

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 29 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB 99 01824

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-30 relate to an extremely large number of possible compounds and methods relating to methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds given Formula I and II in where A is as indicated and/or A' has a group R1 = difluorethyl (CF₂H-CH₂-) or trifluorethyl (CF₃-CH₂-). The search includes the use of the above mentioned compounds, pharmaceutical compositions comprising them, in vitro methods using compositions and methods of production thereof.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT, GB 99/01824

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
| W0 9822496 A | 28-05-1998 | AU 5551098 A EP 0941233 A HR 970618 A US 5866684 A | 10-06-1998 15-09-1999 31-08-1998 02-02-1999 |
| W0 9736587 A | 09-10-1997 | AU 2722197 A CA 2250232 A EP 0906099 A | 22-10-1997 09-10-1997 07-04-1999 |
| W0 9743310 A | 20-11-1997 | AU 2933797 A CA 2254122 A EP 0907659 A | 05-12-1997 20-11-1997 14-04-1999 |



11111

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PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| | | |
|--|---|--|
| Applicant's or agent's file reference KMN/FP5780044 | FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
| International application No. PCT/GB 99/ 01824 | International filing date (day/month/year) 09/06/1999 | (Earliest) Priority Date (day/month/year) 10/06/1998 |
| Applicant ISTITUTO DI RICERCHE DI BIOLOGIA MOLECOLARE..et al | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 29 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-30 relate to an extremely large number of possible compounds and methods relating to methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds given Formula I and II in where A is as indicated and/or A' has a group R1 = difluorethyl (CF₂H-CH₂-) or trifluorethyl (CF₃-CH₂-). The search includes the use of the above mentioned compounds, pharmaceutical compositions comprising them, in vitro methods using compositions and methods of production thereof.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.



| | | | | | |
|---|---|------------|--|------------|-----------------------|
| A. CLASSIFICATION OF SUBJECT MATTER | | | | | |
| IPC 6 | C07K5/06 | C07K5/08 | C07K7/06 | C07C237/22 | C07C311/45 |
| | C07D209/26 | C07D209/32 | C07D307/94 | C07D333/24 | C07D407/12 |
| | C07D409/06 | C07D409/12 | A61K38/05 | A61K38/06 | A61K38/08 |
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | | |
| B. FIELDS SEARCHED | | | | | |
| Minimum documentation searched (classification system followed by classification symbols) | | | | | |
| IPC 6 | C07K | C07C | C07D | A61K | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | | | | |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used) | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | |
| Category * | Citation of document, with indication, where appropriate, of the relevant passages | | | | Relevant to claim No. |
| X | WO 98 22496 A (HOFFMANN LA ROCHE) 28 May 1998 (1998-05-28) page 2, line 10 - line 14; claims; examples 3,8,17-19,47-58,61,62 page 15, line 14 -page 18, line 12 ---- | | | | 1-29 |
| X | WO 97 36587 A (MERCK & CO INC ;HEIMBROOK DAVID C (US); OLIFF ALLEN I (US); STIRDI) 9 October 1997 (1997-10-09) page 298, line 5 - line 6; claims; examples page 299, line 1 - line 2 ---- | | | | 1,26 |
| A | WO 97 43310 A (SCHERING CORP) 20 November 1997 (1997-11-20) claims; examples ----- | | | | 1-29 |
| <input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. | | | | | |
| * Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family | | | | | |
| Date of the actual completion of the international search | | | Date of mailing of the international search report | | |
| 15 September 1999 | | | 22/09/1999 | | |
| Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | | | Authorized officer Fuhr, C | | |

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K31/195 A61K31/335 A61K31/38 A61K31/405

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Further documents are listed in the continuation of box C.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

15 September 1999

Date of mailing of the international search report

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Authorized officer

Fuhr, C

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